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The Effects of Prolonged Standing Compared to Prolonged Sitting on Postprandial Lipemia

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The Effects of Prolonged Standing Compared to Prolonged Sitting on Postprandial Lipemia

by

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Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Kinesiology

The University of Texas at Austin

August 2017

Abstract

The Effects of Prolonged Standing Compared to Prolonged Sitting on Postprandial Lipemia

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The University of Texas at Austin, 2017

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Prolonged periods of sedentary behavior are linked to cardiometabolic disease independent of many risk factors including exercise and activity levels. This study examined the effects of posture, rather than activity by comparing one full day of sitting (14.45 ± 0.286 hours) compared to one full day of standing (12.22 ± 0.105 hours) on postprandial metabolism and lipemia. Eighteen subjects aged 23.72 ± 0.795 years completed two (sit/stand), four-day trials in a randomized crossover design. The first two days of the trial were control days where activity and diet were matched across trials. The third day involved one full day of sitting or standing. The fourth day was a high fat tolerance test, in which blood and gas samples were collected immediately before and for six hours after the ingestion of a high fat shake (1.34, 0.92, 0.17 g/kg body weight of fat, carbohydrate, and protein, respectively). Indirect calorimetry was used to measure metabolic rate and substrate oxidation while spectrophotometry was used to measure plasma concentration. Area under the curve (AUC) for the postprandial responses in plasma glucose and triglyceride were calculated with the trapezoidal rule. Prolonged

standing resulted in significantly lower plasma triglyceride concentration (p=0.036) and an 11.3% decrease in total AUC (p=0.022) compared to sitting, but no change in incremental AUC (AUCi) was detected (p=0.186). There were no changes in substrate oxidation (p=0.522) or plasma glucose glucose concentration (p=0.776) during the high fat tolerance test. The study shows that posture does influence the lipid profile independent of exercise or activity by increasing the clearance of triglyceride from the blood stream without increasing the oxidation of fat.

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GENERAL INTRODUCTION

With the advent of technology in modern times, people find themselves sitting more and more. Self-reports show that Americans sit an average of 8 hours per day, while objective measurements show that Australian workers sit an average of 69.4 percent of waking hours during work days, which would translate to approximately 11.1 hours (Patel et al., 2010) (Hadgraft et al., 2016). Although technological advances have brought countless benefits to modern day life, it would appear that the increased inactivity accompanying this new era is associated with increased mortality risk from all causes, particularly cardiovascular disease (Katzmarzyk et al., 2009). Interestingly, meta-analysis shows that the negative effects of prolonged sitting appear to be independent of the volume of structured exercise, thus suggesting that an individual partaking in extensive periods of inactivity may not reduce health detriment with acute bouts of exercise (Biswas et al., 2015). Because of this, people that meet ACSM (American College of Sports Medicine) guidelines for volume of physical activity may still be putting themselves at risk by sitting too much.

Elevation of blood triglyceride content after eating, known as post-prandial lipemia (PPL) is associated with increased risk of cardiovascular disease and mortality (Nordestgaard et al., 2007). PPL is believed to attribute to atherosclerosis by depositing chylomicron remnants onto arterial walls (Mamo et al., 1998). This has led to the notion that post-prandial lipid metabolism may be used as a marker of cardiovascular health and has spawned much research into interventions to reduce PPL. Previous studies have shown that various forms of exercise, including one hour of running, reduce the PPL response after a high fat meal (Trombold et al., 2013) (Kim et al., 2014). This effect was only observed in an active population taking more than 5,000 steps per day. Furthermore,

prolonged bouts of inactive sitting not only raise PPL, but it also diminishes the healthy effect of acute exercise (Kim et al., 2016). In other words, a person who is engaging in the recommended amount of exercise may not be receiving a major exercise-induced benefit to cardiometabolic health if they spend too much of their non-exercising time seated. There are already many people who do not perform structured exercise, but the notion that prolonged sitting may be independently detrimental to PPL is concerning.

Naturally, one would inquire if the negative effects of prolonged sitting could be attenuated by replacing sitting with standing. Although this seems sensible, objective measures are needed to determine whether prolonged standing improves lipid metabolism before recommendations can be made. In rats, it has been observed that abolishing ambulatory activity results in increased PPL and decreased lipoprotein lipase -- an enzyme responsible for shuttling plasma triglyceride into muscle cells (Bey & Hamilton, 2003). Three human studies have compared sitting and standing but have failed to show significant reductions in PPL (Miyashita et al., 2013) (Thorp et al., 2014) (Henson et al., 2016). These studies, however, required subjects to stand and sit in an intermittent fashion to break up prolonged sitting, rather than entirely replacing sitting with standing. Furthermore, these preliminary studies accumulated only one hour to four-and-a-half hours of standing in total. The current study aimed to examine the changes to PPL when 12 hours of sitting are replaced with 12 hours of standing.

Research Purpose and Hypotheses

This study examines the effect performing one full day of prolonged sitting or prolonged standing on PPL during a high fat tolerance test. The specific hypothesis was as follows:

- Prolonged standing will reduce PPL compared to prolonged sitting.

REVIEW OF LITERATURE

Introduction

Cardiovascular disease has not only become responsible for more deaths in the United States than any other health event and 17% of health care expenditures as of 2011, it is projected to effect 40.5% of Americans by 2030 (Heidenreich et al., 2011). With such an ominous forecast, the discovery of new and effective interventions to reduce the risk of cardiovascular disease has become paramount. It is clear that exercise is a proven method to reduce the risk of cardiovascular disease as well as promote many other health benefits (Pate et al., 1995). More recently, however, it has become apparent that sedentary behavior may not only mitigate exercise induced health benefits, but it may elicit its own independent detriments to health as well (Owen et al., 2010). The average level of physical activity of Americans has dropped considerably since 1950 due to changes in technology and culture (Brownson et al., 2005), which makes sedentary-induced health detriments even more concerning. Investigation into the nefarious effects that prolonged sitting has on health has led to the development of a new field of study deviating from the well-established field of exercise physiology: 'inactivity physiology' (Hamilton et al., 2007).

With the emergence of a new physiological field and a notable increase in sedentary activities, a primary goal is to understand the complex interactions between sedentary behaviors, body posture, non-exercise physical activity, exercise, and health. Through review of epidemiological studies as well as interventional research, one may begin to associate the complicated network of interactions between these variables, and hopefully apply them to promote better advocacy and practical applications to improve the health of many. One such method that may be applied to those affected by the

advancement of workplace technology would be replacing seated time with standing time. If standing rather than sitting helps to promote health benefits, then it may be possible to combat sedentary-induced health detriment without impeding the technological cultural shift that has contributed to reduced physical activity levels.

Section I: Sedentary Behavior and Morbidity

To understand sedentary behavior, one must first define it. Sedentary behavior has been defined by the American Heart Association as activity with an intensity ranging between 1 and 1.5 metabolic equivalents. A metabolic equivalent is the energy expended while resting in a seated position, which results in an oxygen consumption of approximately 3.5 ml of oxygen per kilogram of body weight per minute (Ainsworth et al., 2000). The Sedentary Behavior Research Network included body position in its definition to describe sedentary activities as "any waking behavior characterized by an energy expenditure less than or equal to 1.5 metabolic equivalents while in a sitting or reclining posture" (Sedentary.Behaviour.Research.Network, 2012). Many behaviors that fall under this category are very common in the American lifestyle including sitting during computer use, class work, driving, playing video games, watching television, reading, and social conversation.

Early inquiry into the effects of sedentary behavior on cardiovascular health was primarily epidemiological; the presence of mortality or morbidity due to cardiovascular disease gave a clear and dichotomous measurement of cardiovascular health. The first, and most classic of these sedentary epidemiological studies, was conducted by Jeremiah Morris et al. in 1953; it was discovered that double decker bus drivers incurred a significantly higher incidence of myocardial infarction compared to conductors (Morris et al., 1953). A primary difference between the two professions is the amount of time spent doing sedentary activity. The bus drivers would spend the vast majority of their day seated as they worked, while the conductors were constantly standing, walking, and climbing up and down stairs. This iconic study was the first of many epidemiological

investigations into the relationship between physical activity and cardiovascular morbidity and mortality.

The first challenge when setting up an epidemiological study is determining the method for quantifying the amount of time spent inactive. Morris introduced the idea by using occupation. This provided insight, yet assumed inactivity instead of providing measurements. Subsequent studies would utilize self-report assessments from subjects to quantify sedentary behavior time. However, as expected, the validity of subjects' self-reporting was brought into question. It was found that subjects estimated the time they spend in sedentary activity more accurately when it was attached to television viewing, compared to a less accurate and low estimation of sedentary time regarding more general reports such as time spend performing sedentary behavior in the workplace (Clark et al., 2011) (Thorp et al., 2012). A later study showed a reduced cardioprotective effect of exercise for the same amount of self-reported television viewing compared to self-reported inactivity (Ekelund et al., 2016). The inconsistency of self-reports for sedentary activity compared to television viewing displays a need for more objective measurements of activities. Accelerometers have become the common measurement tool for physiologists studying inactivity. An accelerometer is a small device that can be worn on the waist, wrist, ankle, or leg to measure acceleration. These devices convert movement to electrical signals that are summed to quantify total sedentary time (Healy et al., 2011). Unfortunately, these devices do not give any context to the activity, so a combination of device assessment and self-reporting may be the best combination to paint a true picture.

Even with questions regarding the measurement of activity, it seems clear that prolonged inactivity poses a burden on cardiovascular health. There is a consistent and direct relationship between television viewing time and the presence of cardiovascular disease (Ford & Caspersen, 2012). This relationship is also seen with instances of total

cardiovascular disease as well as incidence of nonfatal cardiovascular disease and coronary heart disease (Stamatakis et al., 2011). Perhaps most interestingly, this relationship persists even when controlled for sociodemographic status, obesity, overall health, and amount of moderate to vigorous physical activity performed (Chomistek et al., 2013). However, the dangers of prolonged periods of sitting do not just stop at cardiovascular disease. Individuals who self-report viewing more than 7 hours of television a day not only display a doubling in risk of cardiovascular mortality, but they also have a 60% greater risk for all-cause mortality and a 20% increase in risk for cancer mortality, when adjusted for quantity of moderate to vigorous physical activity (Matthews et al., 2012).

These epidemiological studies indicate the importance of studying the relationship between inactivity and health, but they are reliant on morbidity or death. Because of this, they are unable to study the effects of inactivity on health in non-diseased individuals prior to morbidity. To better understand sedentary effects before the onset of disease, a measurable proxy for in vivo cardiovascular health became necessary, leading to the use of postprandial lipemia in this area of research.

Section II: Postprandial Lipemia as a Marker of Health

The elevation of blood triglyceride concentration after eating is known as post-prandial lipemia (PPL); it can last for six to eight hours and the degree of this response is associated with increased risk of cardiovascular disease and mortality (Nordestgaard et al., 2007). PPL is measured by calculating the area under the curve of plasma triacylglycerol concentration over the course of 6 to 8 hours post meal ingestion (M. Maraki et al., 2011). When measuring PPL, it is common to also collect gas samples; the collection of these samples allows one to indirectly calculate fat oxidation (Frayn, 1983). As such, a measurement of postprandial fat metabolism through PPL allows insight into cardiometabolic health in living, non-diseased people.

One way that chronically high PPL likely contributes to health is by altering the concentration of high density lipoprotein cholesterol; high concentrations of this cholesterol are associated with reduced risk of cardiovascular disease (Barter & Rye, 1996).The concentration of high density lipoprotein cholesterol is highly dependent on the body's ability to metabolize triglyceride rich lipoproteins, especially in the fed state (Miesenbock & Patsch, 1992). In postprandial lipemia, chylomicrons accumulate from ingested lipids, and these absorbed triglycerides compete with high density lipoprotein cholesterol for clearance from the blood through the same pathway in the liver, which is mediated by lipoprotein lipase (Bjorkegren et al., 1996). Cholesterol from high density lipoprotein is transferred more rapidly to triglyceride rich lipoproteins, thus reducing the high density lipoprotein cholesterol concentration and resulting in an increase in small-dense low density lipoproteins, which are believed to be atherogenic (Griffin, 1997). Because of this, the efficiency of one's body to metabolize fat directly alters this increase in small-dense low density lipoproteins, allowing for measurability of a healthy response.

PPL may also contribute to cardiovascular disease risk through direct deposition of postprandial lipoproteins onto arterial walls, thus causing the buildup of plaque, also known as atherosclerosis (Zilversmit, 1979) (Mamo et al., 1998). Further arterial health outcomes are observed as ingestion of fat increases blood coagulability through a rise in thrombotic plasma factors and induces endothelial dysfunction hours after feeding, which is a component of the atherosclerotic process (Vogel et al., 1997) (Miller et al., 1991).

The relationship between high concentrations of postprandial lipoproteins and arterial plaque accumulation is well established (Karpe, 1999). Therefore, it would seem reasonable to use meal-induced PPL and the liver output of postprandial lipoproteins and triglycerides as a measurable surrogate for cardiometabolic health in research studies.

Section III: Effects of Exercise on Postprandial Lipemia

If physical inactivity is culpable for cardiovascular disease risk, one would naturally inquire as to how physical activity may mitigate this risk, especially when activity is amplified to the degree of exercise. The use of oral fat tolerance tests to measure PPL is now widespread throughout exercise physiology research, and much has been elucidated as to the relationship between exercise and PPL. In general, acute exercise results in a reduction of PPL, but this is influenced by variables such as oral fat tolerance test timing, intensity and volume of exercise, energy expenditure and balance, macronutrient content of prior feedings, training status, and prior bouts of prolonged sedentary activity.

If oral fat tolerance tests are to be used in conjunction with exercise for comparisons, the first and most obvious question is when to test subjects. Should test meals be ingested prior to the exercise bout? After, perhaps? And if so, how long after is ideal?

When exercise is performed immediately after the ingestion of the test meal results are unclear. Some studies show that there is a reduction in PPL after exercise compared to control groups, while others show no apparent difference (H. Cohen & Goldberg, 1960) (Sady et al., 1986) (Zhang et al., 1998) (Welle, 1984). When no meal is given, there is either no change or an increase in the fasting plasma triglyceride concentration immediately following a bout of exercise (Thompson et al., 1980) (Dufaux et al., 1986). A clear reduction in PPL is present when a test meal is given within one hour after a bout of aerobic exercise ranging from 60-70% of maximal oxygen consumption for at least 50 minutes (Katsanos et al., 2004) (Plaisance et al., 2008). This immediate PPL reducing response is not seen with very low aerobic intensities of 25%

VO2max, and with resistance exercise, PPL is exacerbated (Burns et al., 2006; Katsanos et al., 2004). These test meals had a high composition of fat, but there appears to be no clear PPL response when the test meal has a moderate fat content (Pfeiffer et al., 2005) (Cox-York et al., 2013). Many athletes, recreational and competitive, consume meals immediately after training sessions, thus denoting the importance of these findings.

Reductions in PPL are more clear and robust, however, when the test meal is ingested 12 to 24 hours after the bout of exercise where a reduction in PPL is present in continuous aerobic exercise, intermittent sprint exercise, intermittent aerobic exercise, and resistance exercise (Herd et al., 2001) (Gill et al., 2001) (Freese et al., 2011) (Altena et al., 2004) (Zafeiridis et al., 2007). Similar to immediate meal ingestion, this response does not occur at very low intensities like slow walking (Tsetsonis & Hardman, 1996a) (Petitt et al., 2003). The exercise-induced reduction in PPL disappears sometime between 24 and 40 hours after the exercise bout (Pafili et al., 2009). Because of the timing of this response, and its largest reductions happening hours after exercise, it can be implied that exercise may induce some change to fat metabolism different than just altering blood flow and substrate ability.

It is observed that most forms of exercise reduce PPL in oral fat tolerance tests conducted the day after exercise, but the magnitude of reduction is not the same across all forms, volumes, and intensities.

When observing continuous aerobic exercise, greater intensities elicit greater effects on PPL compared to lower intensities when volume is controlled (Malkova et al., 1999) (Tsetsonis & Hardman, 1996a). In fact, PPL was not reduced at all when walking for 90 minutes or jogging for 30 minutes (Petitt et al., 2003) (Altena et al., 2004). Interestingly, when matched for energy expenditure, 180 minutes of walking at 30% VO2max causes the same percent reduction in PPL as 90 minutes of running at 60%

VO2max (Tsetsonis & Hardman, 1996b). This would imply that energy expenditure is a primary variable determining the effectiveness of exercise at reducing PPL and that there may be an energy cost threshold which must be reached before protective cardiometabolic effects occur.

However, this is only seen when matching energy costs for low intensity exercise compared to moderate intensity exercise. When comparing continuous aerobic exercise of moderate intensity (one hour at 50%VO2peak) to high intensity aerobic interval exercise (5 minute intervals at 90% VO2peak), the results are not equal even though energy expenditure was the same (Trombold et al., 2013). The high intensity aerobic interval exercise was significantly more effective at reducing PPL. PPL reductions are also seen in sprint interval exercise accumulating in 2 minutes of sprinting over the course of 18 minutes, even though the energy expended is lower than continuous aerobic exercise studies which did not show an effect (Freese et al., 2011). Therefore, it seems that although energy expenditure is an important component in altering PPL in moderate intensity exercise and lower, either intensity or the type of exercise – intermittent vs continuous – influences the efficacy of exercise-induced PPL reduction when the relative work load is very high.

Energy expenditure is not the same as energy balance. The studies that used continuous aerobic exercise did not compensate for the energy expended with increased food consumption. Therefore, subjects were in a hypocaloric state, which may be a causative factor for PPL reduction independent of the exercise itself. In fact, when energy balance is restored after a bout of walking at 50% VO2max, there is no longer a protective PPL response, which is present when energy balance remains negative (Burton et al., 2008).

To further investigate, one group examined the effects of exercise on PPL compared to diet-induced energy deficit. Although the exercise and diet involved the same amount of caloric deficit, exercise was significantly more effective at reducing PPL (Gill & Hardman, 2000). This could be due to several reasons. It is possible that there is a caloric deficit threshold, which is higher for diet induced negative energy balance compared to exercise. It is also possible that diet and exercise reduce PPL through different mechanisms or that energy deficit is only one of several factors contributing to exercise induced PPL reduction (M. I. Maraki & Sidossis, 2013).

As previously discussed, intensity is likely one of those factors, which becomes increasingly interesting when paired with caloric balance. Low intensity walking exercise reduces PPL when combined with diet compared to walking without energy restriction; this walking is normally too low of an intensity to elicit a PPL effect (M. Maraki et al., 2009) (Tsetsonis & Hardman, 1996a). At these low intensities or with diet alone, an energy deficit of approximately 30 kJ/kg body weight was needed for a beneficial metabolic response, but a smaller energy deficit was needed for resistance exercise (M. Maraki & Sidossis, 2010). Exercise maintains a reduction in PPL at high intensities regardless of caloric balance. Four bouts of maximal effort sprint interval exercise reduces PPL in the eucaloric state but is attenuated compared to negative energy balance (Freese et al., 2011).

The macronutrient content of energy compensation also likely plays a role in this response. When subjects perform a combination of 60 minutes of moderate intensity (65% VO2peak) and ten high intensity aerobic intervals (2 minutes at 80%-90% VO2peak), PPL is reduced in the eucaloric and hypocaloric states (Trombold et al., 2014). Interestingly, when the compensation meal contained a high carbohydrate content, the response was mitigated, but when the compensation meal contained a low

carbohydrate content, the response was the same as exercise without energy compensation. Energy balance clearly plays a role in the PPL response, but it appears to be less impactful when exercise is of high intensity or when the exerciser maintains a negative carbohydrate balance, despite maintaining neutral caloric balance.

Exercise poses a marked influence on cardiometabolic health, but many of the studies discussed describe acute bouts of exercise. Is the benefit of exercise caused only by acute bouts, or is there a healthy training effect resulting from chronic adaptation? Cross-sectional analysis shows that athletes have a smaller PPL response than sedentary subjects, which may lead one to believe that there is a cardiometabolic benefit to long term exercise training independent of each individual bouts of exercise (J. C. Cohen et al., 1989). This conclusion is questionable, however, because subjects were asked to continue their typical training program during the days leading up to the oral fat tolerance test. Another study showing similar results asked subjects to abstain from exercise for 12 to 36 hours before testing (Merrill et al., 1989). It is likely that highly trained athletes would exercise unless explicitly asked not to, which makes it possible that their most recent training bout was still eliciting effects seen with any acute bout of exercise. This would prevent any true insight into the effects of chronic training independent of acute exercise.

The same problem is observed when intervention studies put subjects through periods of endurance training and conduct oral fat tolerance tests within 36 hours of the most recent exercise bout (Weintraub et al., 1989). When male distance runners were asked to refrain from exercise for 14 to 22 days, a subsequent oral fat tolerance test resulted in a 40% increase in PPL (Mankowitz et al., 1992). A 35% increase in PPL is observed in endurance athletes when exercise is absent for 6.5 days prior to testing compared to 15 hours prior (Hardman et al., 1998). Therefore, evidence would suggest

that the cardiometabolic health benefits induced by exercise are not enhanced by chronic training and require a recent, acute bout of exercise.

This is not to say that training has absolutely no benefits to the PPL response in any way, though. Exercise training increases functional capacity and allows one to expend more energy for the same relative intensity compared to untrained individuals. Because of this, exercise training will allow one to increase the volume and intensity of an individual exercise bout, thus increasing the PPL response indirectly.

Although these studies show that exercise poses the ability to benefit cardiometabolic health, it has been seen that this effect can be dampened, or even cancelled when paired with an overly sedentary lifestyle. When observing individuals engaging in greater than 35.5 MET-h/week of activity, those who watch television for more than 5 hours per day exhibit a significantly higher hazard ratio for cardiovascular disease than those who watch 4 hours or fewer (Ekelund et al., 2016). This trend is seen not only in these highly active people, but also in all quartiles of physical activity used in the meta-analysis including 30, 16, and less than 2.5 MET-h/week. Furthermore, data has shown that when an individual takes approximately 1,650 steps per day and maximizes sitting for four days, any PPL reduction from an hour run at 67% of maximal oxygen consumption disappears, even when a eucaloric state is induced (Kim et al. 2016). The exercise-induced reduction in PPL is restored when activity is increased to 17,000 steps per day and sitting is minimized. This effect has been coined ‘Exercise Resistance’, and poses many new questions surrounding the effects that sedentary lifestyles may have on the health benefits of exercise.

Section IV: Standing and Postprandial Lipemia

Although exercise elicits clear benefits to health, many people choose to not partake. For those who would rather not exercise, non-exercise physical activity may still benefit them. The United States has decreased employment for active occupations like agriculture as the technology and service industry has expanded, thus resulting in increased amounts of work time dedicated to sitting rather than physical activity; this has decreased occupational energy expenditure by approximately 100 kilocalories per day compared to 50 years ago (Church et al., 2011). In fact, objective measurements show that a typical Australian worker spends approximately 78.8% of their time at work sitting, resulting in 69.4% of their total waking hours in a chair (Hadgraft et al., 2016). Therefore, it is imperative to try to find ways to improve cardiometabolic health in individuals with low exercise adherence. One such answer may be the use of body posture, i.e. standing vs sitting, to break up or replace prolonged bouts of sedentary behavior.

The advancement of accelerometer and inclinometer technology has increased battery life and allowed these devices to continuously collect data over several days without replacement. This has allowed researchers to measure bouts of sitting, standing, and activity objectively in both laboratory and long-term, free-living conditions with accuracy (Steeves et al., 2015).

One free-living study observed 31 individuals over the course of three months using an activPAL activity monitor and gave 18 of them a sit/stand desk to use freely; they observed a sustained two hour decrease in sitting time for the group given the desks and found them to have higher fasting plasma concentrations of high density lipoprotein cholesterol (Alkhajah et al., 2012). Another study used cross-sectional analysis to

compare 168 different people in their free-living conditions and showed that individuals who interrupted prolonged sitting bouts more often showed healthier cardiometabolic measurements including body mass index, waist circumference, and fasted plasma glucose and triglyceride concentration; this was completely independent of the total amount of sitting, exercise, and intensity of the interruptions (Healy et al., 2008). Per this finding, the composition and duration of sedentary bouts would appear to be a contributing factor to sedentary activity's cardiometabolic effect. Breaking up the prolonged periods of sitting often seen in the workplace may be a simple and effective method of incorporating healthier habits in individuals who refuse to exercise, but investigation with tighter controls than cross-sectional, free-living analysis is needed to confidently propose this idea.

Several interventional studies have tried to remain relevant to the work environment by implementing practical, one-day protocols that could easily be replicated in the daily lives of an office worker, while maintaining laboratory controls and crossover study designs. When comparing 5 hours of uninterrupted sitting to 5 hours of sitting interrupted by 2-minute standing bouts every 20 minutes, there is no clear effect on resting plasma triglycerides even though plasma glucose concentration was reduced in the hours following ingestion of a test meal (Bailey & Locke, 2015). This observation only involved 28 cumulative minutes of standing, however, and used measurements of plasma glucose rather than PPL. When the prolonged sitting bout was increased to 7.5 hours one day and then compared to the same sitting time broken up with 5 minutes of standing every 30 minutes, a reduction in plasma glucose is observed, but there was no reduction in PPL for the three hours following a standardized breakfast and three hours following the same meal for lunch (Henson et al., 2016). It should be noted that the subjects in question were all overweight/obese, postmenopausal, dysglycemic women,

which very well could have altered responses the protocol could have achieved with a non-pathological population. These studies only accrued 28 minutes and 40 minutes of standing, respectively, over the course of a one day protocol, which seems trivial in comparison to the two hours of reduced sitting seen in the free-living study discussed above. Furthermore, although food consumption was recorded, it was not controlled for specific energy balance or deficit. A similar study in young, healthy, normolipidemic males compared six, 45 minute periods of standing separated by 15 minutes of sitting to 7 hours of continuous sitting; even though standing time increased to 4.5 hours, there was not an apparent change in the PPL response, despite reductions in plasma glucose after an oral fat tolerance test of moderate fat content. (Miyashita et al., 2013). Low volume walking in the same experiment did, however, elicit a PPL response. Again, food consumption was recorded and replicated between all trials but did not control for energy deficit or balance, and in this study, the test meal was of moderate fat content.

As of now, one day interventional studies in laboratories have failed to show the benefits of standing that have been observed in longer term free-living, cross-sectional investigations. It is possible that single days of increased standing may still be of value, however, only if standing time is increased, energy balance is manipulated, or a combination of the two.

Longer-term laboratory interventions are more difficult to implement, but one study has explored the effects of standing versus sitting in a controlled laboratory setting over the course of five days. Overweight and sedentary Australian workers used a sit/stand desk to accomplish office work in the laboratory over the course of two 5-day trials in which they continuously sat for 8 hours a day or alternated 30 minute bouts of standing and sitting over 8 hours, accumulating to 4 hours per day of standing (Thorp et al., 2014). This time, diet was manipulated to induce energy balance every day. Similar to

the other interventional studies, there was a reduction in blood glucose with standing, but there was not a change in four hour PPL between the two protocols, thus still failing to show the standing benefits seen in cross-sectional analysis.

The discussed interventional studies failed to replicate the PPL benefits that would be expected based on the free-living studies, which could be due to several reasons. It is possible that the free-living interventions had underlying confounding variables with the lack of a controlled laboratory setting. Because the free-living studies were cross-sectional as opposed to crossover designs, the lifestyles, hormonal profiles, and many other physiological factors relating to the bodies and health of the subjects could have simply been different. It is also possible that energy balance plays a key role in PPL reduction for an activity with as low of an intensity as standing. Energy expenditure is raised by 11.5% when standing continuously for one hour compared to sitting (Gibbs et al., 2016). This means that 4 hours of continuous standing would only increase energy expenditure by 48 kilocalories in the average American woman and 57 kilocalories in the average American man (Fryar et al., 2012). With energy expenditure so low, it is reasonable to believe that standing may contribute to a healthier cardiometabolic response when combined with diet-induced energy deficit. It should also be noted that all studies utilized measures to break up prolonged bouts of sitting but did not completely replace sitting with standing. It is possible that a PPL reduction may be observed when standing is present in more prolonged bouts where accumulative standing is greater than 4.5 hours – which was the highest seen in any study – or when standing occurs in the absence of sitting throughout the day.

Summary

Cardiometabolic health involves the interplay of many different health variables, but it is clear that sedentary activity poses health detriments independent of common cardiovascular disease risk factors such as obesity, overall health status, sociodemographics, and BMI (Chomistek et al., 2013). By using the PPL response following an oral fat tolerance test, researchers are able to investigate these influences prior to the induction of a diseased state. Unsurprisingly, exercise has the ability to increase cardiometabolic health, but this is influenced by the exercise modality, intensity, energy balance and expenditure, macronutrient composition, and even overall sitting time. For those who do not want to participate in exercise to increase their health, alterations in their body posture pose possible alternatives. Studies that break up prolonged sedentary bouts with standing seem beneficial in free-living observational and interventional scenarios but are discordant with laboratory interventions regarding lipid concentration measurements. With relatively few analyses in this particular area, there is a need for further research with greater standing quantities, emphasis on the state of energy balance, and the replacement of sitting rather than fragmentation of prolonged bouts.

STUDY: THE EFFECTS OF PROLONGED STANDING COMPARED TO PROLONGED SITTING ON POSTPRANDIAL LIPEMIA

Introduction

As the industrialized world continues to make technological advancements, the amount of time spent engaging in prolonged periods of sitting has vastly increased. Epidemiological analysis has pointed to prolonged sitting as a risk factor for cardiovascular disease, independent of many other common risk factors(Biswas et al., 2015). Therefore, interventions that reduce this sedentary time may prove beneficial to health. The degree of postprandial lipemia (PPL), or the rise in plasma triglycerides in the 6 to 8 hours after feeding, is associated with cardiovascular disease and atherosclerosis and is consequently often used as a surrogate marker of cardiovascular health in research(Nordestgaard et al., 2007). It is established that exercise of many forms induces a reduction in PPL(Gill et al., 2001; Herd et al., 2001), but it has recently been found that this effect can be nullified by large quantities of prolonged sitting during non-exercising waking hours(Kim et al., 2016).

Because prolonged sitting increases cardiovascular disease risk independently and can even prevent health benefits of exercise, it is practical to investigate the effects of reducing prolonged sitting. Standing is an attractive alternative to sitting because it would not prohibit many of the activities involved with sitting, and could be used for populations with low exercise adherence. Several studies have broken up prolonged periods of sitting with standing resulting in reductions to blood glucose or insulin, but not to PPL(Bailey & Locke, 2015; Henson et al., 2016). This study aimed to completely replace one 12 hour day of prolonged sitting with standing, rather than fragmenting these

periods. Controlled feeding ensured that energy intake matched resting metabolism. The purpose of this design was to utilize increases in PPL to determine if body posture is a contributing and manipulative variable that may be altered to incite a healthier response. We hypothesized that one day of prolonged standing would reduce PPL compared to one day of prolonged sitting.

Methods:

Research Participants

Eighteen healthy, recreationally active but untrained, males and females (8 males, 8 females) aged 23.72 ± 0.795 years completed two different trials in a randomized, crossover experimental design. Trials were separated by at least six days. Subjects body mass 79.06 ± 4.988 kg, their height was 174 ± 2.805 cm, and their BMI was 25.75 ± 1.137 kg/m^2. One subject displayed both an obese BMI and plasma nutrient concentration, but statistical analysis did not gain or lose significance with the obviation of this subject's data. Subjects had no history of cardiovascular or metabolic disease, and were all non-smokers. Participants signed their informed consent prior to participation, and were informed of all possible risks and procedures involved with the study. This study was given ethical approval from the University of Texas Institutional Review Board.

Experimental Design

The protocol consisted of preliminary testing, followed by two phases that each were performed twice: a controlled activity phase of two days, a standing/sitting intervention phase of 12 hours on the third day, and a high fat tolerance test phase lasting 6 hours on the third day. Subjects were asked to refrain from exercise from the start of the first controlled activity day to the end of the high fat tolerance test. There was a minimum 6-day washout between trials. Visual representation of the study design can be found in Figure 1.

Preliminary Testing

Prior to any trials, each subject came to the laboratory for preliminary testing the morning after an 8 hour fast. During this time, subjects sat for 30 minutes, then stood for 30 minutes; subjects did so while breathing into a meteorological balloon. Samples from the balloon were analyzed with a mass spectrometer and volume analyzer so that resting and standing metabolic rates can be measured. Metabolic measurements were used to determine the caloric content of the provided meals. Subjects' weight and height were measured prior to metabolic testing. Subjects were also asked to complete a health history questionnaire and informed consent form prior to any measurements.

Controlled Activity Phase

The controlled activity phase consisted of the two days prior to the sitting/standing intervention. Subjects arrived at the laboratory at approximately 8:00 hours. They were given a pedometer and were asked to be aware of their step count, and to take 5,500-6,500 steps per day, which is concordant to a non-sedentary, low level of physical activity (Tudor-Locke & Bassett, 2004). Subjects were also equipped with an activPAL activity monitor on a randomly chosen thigh, which was used for the monitor across both trials. The activity monitor is a small flat device (approximately 3.3 cm) which was secured to the thigh via Tegaderm. It is equipped with both an accelerometer and an inclinometer that allows it to measure body position and movement, and estimate intensity of activity and energy expenditure. Subjects were asked to eat their normal diet and to log everything consumed and the time at which it was consumed into a provided food journal; they were also asked to take pictures of the food during the two days. Subjects were asked to repeat food consumption exactly during the second controlled

activity phase. Subjects recorded the time that they went to sleep, and repeated this during the other trials.

Sit/Stand/ Intervention Phase

On the day of the sit or stand intervention phase, subjects reported to the Human Performance Laboratory at 8:00 hours following an eight hour fast. The pedometer was removed at this time. They were provided breakfast then asked to stand on a 6 square foot cushioned mat or sit in a cushioned chair for 12 hours total – a time frame slightly longer than estimates of Australian workers mentioned prior (Hadgraft et al., 2016). Sitting and standing were interrupted only for visits to the toilet, but steps were minimized otherwise. When standing, the subjects were allowed to lean on the desk holding their screen. Water was provided ad libitum. They were provided lunch, a snack, and dinner. Provided food contained a macronutrient content of the western variety, containing 59.43% carbohydrate, 19.61% protein, and 20.96% fat. The number of calories provided equaled their resting metabolic rate estimated from preliminary testing, and was replicated for both trials. This induced near energy balance in the sit trial, and slight negative energy balance in the stand trial.

After the 12-hour protocol, subjects were asked to continue wearing the activity monitor, and to fast until returning to the laboratory the subsequent morning for an oral high fat tolerance test. They were asked to lay down when they return home from the laboratory, and to minimize sitting or standing in the time between the intervention and the high fat tolerance test the next morning.

High Fat Tolerance Test Phase

The morning after the standing/sitting intervention phase, subjects arrived to the laboratory to begin the high fat tolerance test phase for measurement of PPL. Upon arrival, the activPAL activity monitor and pedometer were removed, followed by measurement of body weight. Subjects were then seated comfortably in a chair while a catheter was inserted into an antecubital vein. A saline flush was attached to the line and flushed every seven minutes to prevent clogging. After five minutes, a fasted blood sample was collected in a 6 mL K2 EDTA vacutainer, which was promptly centrifuged at 3,000 rpm for 15 minutes at 4°C. Plasma was then aliquoted into an Eppendorf safe-lock tube, labeled, and stored at -80°C until later analysis. This process was repeated for all collected samples.

Once subjects had remained seated for 20 minutes, they were asked to breathe into a meteorological balloon for 10 minutes. This sample was analyzed by a mass spectrometer and volume analyzer to measure fat oxidation and metabolic status.

After the gas sample was taken, subjects were given a high fat shake consisting of melted ice cream and heavy whipping cream (1.34 g/kg fat, 0.92, g/kg carbohydrate, and 0.17 g/kg protein). Subjects were asked to consume the shake within five minutes. After completion of the shake, 6 ml of blood were sampled after 1, 2, 3, 4, 5, and 6 hours. To measure postprandial fat oxidation and metabolic status, ten minute gas samples were also collected after 2, 4, and 6 hours after shake ingestion. Following the 6 hour blood and gas sample, subjects were allowed to leave the laboratory.

Plasma triglyceride and glucose levels were measured enzymatically using commercially available diagnostic kits.

General Measurements and Instrumentation

Resting/Standing Metabolic Rate

On the day of preliminary testing, subjects were asked to sit down for 30 minutes and then to stand for 30 minutes. During the last ten minutes of sitting and standing subjects breathed into a meteorological balloon. Expired air was analyzed with a mass spectrometer (Perkin-Elmer MGA 1100, St Louis, Missouri)and a volume analyzer (Vacumed, Ventura, CA) to find the sitting and standing metabolic rates of the subject through indirect calorimetry. These measurements were used and extrapolated to the number of calories provided during the sit/stand intervention phase.

Postprandial Gas Exchange

During the high fat tolerance test, ten minute gas samples were collected at baseline, 2, 4, and 6 hours post shake ingestion. These samples were analyzed with the mass spectrometer and volume analyzer to determine fat oxidation and metabolism during the test.

Anthropometric Measurements

Body weight was measured using a (Lifesource UC 321 precision scale); height was measured using a physician's scale. Both were measured prior to preliminary testing and the high fat tolerance test.

Diet

During the controlled activity phase, diet was logged and replicated by the subject during subsequent trials. Diet during the sit/stand intervention day was provided by the laboratory. Calories of the diet approximately replicated the subject's resting metabolic rate. This was approximated from the metabolic rates measured during preliminary testing. Macronutrient content of the provided food contained 20.96% fat, 59.43% carbohydrate, and 19.61% protein. On the day of the high fat tolerance test, subjects were provided with a shake consisting of melted ice cream and heavy whipping cream. Macronutrient content of the test shake was 1.34, 0.92, 0.17 g/kg body weight of fat, carbohydrate, and protein, respectively. This resulted in a caloric content of 15.8 kcal/kg body weight (an average of 1249 kcal).

Physical Activity Monitoring

Subjects wore an activPAL activity monitor (PAL Technologies Limited, Glasgow, UK) from the beginning of the controlled activity phase to their arrival at the laboratory for the high fat tolerance test. This device has both an accelerometer and an inclinometer, and was used to measure body position, and movement, and intensity of activity.

The activPAL also measured the number of steps taken; however, subjects were unable to see their step count by this device. Because of this, subjects were also equipped with a pedometer (Yamax Digi-walker CW-701) so that the number of steps taken during the controlled activity phase could be measured with visual feedback. Subjects were asked to take no more than 8,000 steps per day during this period.

Blood Sampling and Preparation

Subjects were fasted for 12 hours prior to the high fat tolerance test. Blood samples were acquired through an antecubital venous catheter. The first sample was taken prior to shake ingestion, and 6 subsequent samples were taken each hour after shake ingestion..

Once blood samples were collected, they were centrifuged for 15 minutes at 3,000 rpm and 4°C. They were then immediately transferred to test tubes and labeled with the identification number of the subject. Samples were then stored in a locked laboratory freezer at -80°C until further analysis.

Blood samples were analyzed for plasma triglyceride and glucose concentration enzymatically using standardized, commercially-available diagnostic kits (Pointe Scientific, Inc. Canton, USA) and a plate reader (Tecan Infinite 200 PRO, Tecan Group Ltd., Mannedorf, Switzerland).

Plasma Triglyceride Measurement

Spectrophotometric methods with commercially available kits and a plate reader were used to measure plasma triglyceride concentration. First, plasma samples were removed from the -80°C freezer and placed in an ice-water bath to thaw. After thawing, 3 µL of plasma were removed and added to 300 µL of triglyceride reagent. The triglycerides (TG) were hydrolyzed by lipase into glycerol and fatty acids; the glycerol was then phosphorylated via ATP into glycerol 3-phosphate (G-3-P) and ADP via glycerol kinase (GK). Glycerol 3-phosphate was catalyzed by glycerophosphate oxidase (GPO) into dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2). The produced hydrogen peroxide then reacted with 4-aminoantipyrine and 3-hydroxy-2,4,6-tribomobenzoic (TBHB) to form a red quinoneimine dye in a reaction catalyzed by

peroxidase. The dye's absorbance was then spectrophotometrically measured at 480 nm. The produced color's intensity is directly proportional to the triglyceride concentration of the sample. Chemical Reactions for this process can be seen in Figure 2.

Plasma Glucose Measurement

Spectrophotometric methods with commercially available kits (Pointe Scientific, Inc. Canton, USA) and a plate reader were used to measure plasma glucose concentration. First, plasma samples were removed from the -80°C freezer and placed in an ice-water bath to thaw. After thawing, 5µL of plasma were removed and added to 1,000 µL of glucose reagent, which was then incubated for 3 minutes at room temperature. The glucose was phosphorylated with ATP, thus producing glucose 6-phosphate (G-6-P) in a reaction that is catalyzed by hexokinase (HK). Glucose 6-phosphate dehydrogenase (G6PDH) then catalyzed a reaction in which glucose 6-phosphate is oxidized and NAD is reduced to form NADH, 6-phosphogluconate, and H^+. NADH absorbance was measured at 340 nm. The NADH concentration is directly proportional to the sample plasma glucose concentration. Chemical Reactions for this process can be seen in Figure 3.

Proposed Statistical Analysis

Total and incremental areas under the curve for plasma triglyceride and glucose concentration were calculated using the trapezium rule. Total postprandial substrate oxidation of carbohydrate and fat, total standing time, total sitting time, daily step numbers, caloric intake, sitting and standing metabolic rate, and fasting plasma concentration of triglycerides and glucose were analyzed using paired samples T-tests.

Postprandial absolute and relative substrate oxidation, and concentrations of plasma triglyceride and glucose were analyzed using two-way ANOVA with repeated measures (trials and time). When interactions were significant, Bonferroni multiple comparisons analyses were conducted. Data were analyzed using Graphpad Prism software (Graphpad Software, San Diego, CA). Data are presented as means and standard error. The level of significance was set at $p<0.05$.

Results

Changes in Body Mass

Subject body weight during preliminary testing, the sitting intervention's high fat tolerance test (HFTTsit), and the standing intervention's high fat tolerance test (HFTTstand) was 79.06 ± 21.08, 78.76 ± 21.27, and 79.02 ± 21.13 kg, respectively. There were no significant differences in body weight between the three trials ($p=0.752$).

Energy Intake

Caloric intake did not change during the sitting and standing intervention days (2131 ± 427.4 kcal/day), as the same food was provided during both trials to match estimated resting metabolism (2118 ± 426.5 kcal/day) found through preliminary testing. During the standing intervention subjects ate 85.58 ± 120.9 fewer kcals than their estimated metabolic rate with 12 hours of standing (2216 ± 500 kcal/day). Taller subjects had greater differences in energy balance on the standing intervention day; there was a significant correlation between height and the difference between estimated sitting and standing metabolism ($r=0.784$, $p<0.0001$).

Steps and Posture Distribution

There was no significant difference in the number of steps that participants took on control days for the sitting trial compared to those for the standing trial ($p>0.05$). Likewise, there was no significant difference in the number of steps that participants took on the intervention day for the sitting trial compared to those for the standing trial ($p>0.05$) (Figure 4; Table 5). There was no significant difference in the amount of time that participants spent standing on control days for the sitting trial compared to those for the standing trial ($p>0.05$) (Figure 5; Table 6).

All measurements of sitting, standing, and stepping on the day of the sit/stand intervention include the entirety of the day, not just the time spent in the laboratory. There was no significant difference in steps taken between the sitting or standing intervention day ($p>0.05$) (Figure 4). As expected, subjects sat significantly more during the sitting trial ($p<0.001$) and stood significantly more during the standing trial ($p<0.001$) (Figure 6). There was no significant difference in time spent stepping between the two trials ($p>0.05$).

Postprandial Metabolism

Indirect calorimetry was used for all measurements of substrate oxidation and metabolic rate. All subjects that displayed a respiratory exchange ratio greater than 1 at any point of the tolerance test were eliminated from gas analysis to control for hyperventilating subjects. Relative fat oxidation includes the percentage of energy derived from fat, relative to carbohydrate. There was no significant difference between trials in relative fat oxidation ($p>0.05$) (Figure 7) or in absolute fat oxidation, ($p>0.05$) (Figure 8). When only examining postprandial values after baseline, there was no significant difference in fat oxidation between trials ($p>0.05$); when only examining fasted baseline values, there was no significant difference in fat oxidation between trials ($p>0.05$).

When comparing baseline and postprandial samples there was no significant difference in metabolic rate between the sitting or standing high fat tolerance test ($p>0.05$) (Figure 9). However, when only examining the postprandial response there was a significant effect of posture on metabolic rate between the sitting and standing trials ($p=0.032$) (Figure 10). When only examining fasted values, there was no significant difference in metabolic rate found between the trials ($p>0.05$).

Plasma Concentrations

After calculating the area under the curve (AUC) using the trapezoidal rule, the HFTTstand total AUC for plasma triglyceride was significantly lower than the HFTTsit total AUC for plasma triglyceride ($p=0.022$) resulting in an 11.3% decrease in total AUC, but it was not significantly higher when comparing plasma triglyceride incremental AUC (AUCi) ($p>0.05$) (Figures 12 and 13). Plasma triglyceride concentrations displayed a significant time effect over the course of the HFTTs ($p<0.001$), as well as a significant posture effect ($p=0.036$), (Figure 11). There was no significant difference in fasted plasma triglyceride concentration between the trials ($p>0.05$).

For plasma glucose, there was no significant difference in total AUC ($p>0.05$), and there was also no significant difference in AUCi between trials ($p>0.05$) (Figures 15 and 16). There was a significant main effect of time on plasma glucose ($p<0.001$), but no significant main effect of posture ($p>0.05$) (Figure 14). Likewise, the intervention did not induce a significant change in fasted plasma glucose levels ($p>0.05$).

Discussion

The primary finding of this study is that one full day of prolonged standing reduces the rise in plasma triglyceride concentration compared to one full day of prolonged sitting. Interestingly, although the total response was reduced, the plasma triglyceride concentration was not statistically different in the fasted state or the four hours after test meal ingestion. The prolonged standing induced a decrease in plasma triglyceride in the fifth and sixth hour after the meal. Prolonged standing also led to a decrease in postprandial energy expenditure compared to sitting by a marginal 31.78 ± 44.73 kcal over the six hours of the tolerance test. No other metabolic markers including plasma glucose concentration, metabolic rate, percent of kcal from fat, or fat oxidation.

Prolonged sedentary activity across a range of time frames has shown to lead to a negative effect on postprandial metabolism by increasing plasma triglyceride concentration. It was found that one month of bed rest increases plasma triglyceride in the fasted and the postprandial state (Bergouignan et al., 2006; Bergouignan et al., 2009). Later, it was discovered that postprandial triglycerides can increase after two weeks of sedentary activity, even though subjects lost lean and total body mass, indicating an independence from positive energy balance (Olsen et al., 2008). Further reducing the sedentary time, Kim et al showed that plasma triglyceride can increase compared to an active (16,000 steps/day) baseline high fat tolerance test after just two days of prolonged sitting by reducing steps to 2000 steps/day (Kim et al., 2016). In this study, four total days of sedentary activity was enough to increase postprandial lipemia despite an hour of moderate intensity exercise, or a 628 kcal deficit the day prior to the high fat tolerance test. There was also no difference between sedentary individuals on a hypercaloric or eucaloric diet. This is observed despite the multitude of studies linking exercise bouts to

improved postprandial lipid profiles (Altena et al., 2004; Gill et al., 2001; Malkova et al., 1999) (Herd et al., 2001). Because the four days elicited no change in mass, it is likely that the postprandial responses following inactivity studies can be linked to the inactivity itself, rather than a loss of muscle mass inducing decreased muscle metabolic function.

With the evidence of sedentary-induced detriments to postprandial metabolism, one may wonder if the cause is simply a lack of contraction and activity over the prolonged period of time, or if it is the posture itself and the act of sitting down that alters postprandial lipemia. This study tries to illuminate the answer by utilizing prolonged standing. Subjects did not take a significantly different number of steps between the sitting or standing trials, thus showing changes independent of actual movement. Furthermore, subjects spent 85.58 ± 120.9 kcal more when standing which is below the range of 200-250 kcal – the lowest amount of energy expenditure needed to observe changes in postprandial lipemia (Miyashita, 2008; Miyashita et al., 2008). Only four subjects spent over 200 kcal more in the standing day than the sitting day; interestingly, all four of which are at least 185 cm tall. With a significant correlation between height and the difference between sitting and standing metabolic rate ($p<.0001$), it should be noted that taller people may gain some benefit to postprandial metabolism through energy expenditure as well as posture when standing for prolonged periods of time. With little movement and energy expenditure, observations in this study focus on the metabolic effects of posture rather than changes to the well-studied exercise-induced benefits.

The present study shows that prolonged standing does decrease the total area under the curve of a high fat tolerance test, but not the incremental area under the curve or any measure of fat oxidation. Incremental area under the curve is often used in postprandial metabolic research because of its accuracy in describing the postprandial triglyceride response to an oral fat load, while total area under the curve is descriptive of

the total lipid profile rather than the postprandial response alone, and more dependent on the fasted triglyceride while incremental is not at all (Carstensen et al., 2003). Incremental area under the curve only includes the area above baseline, so it is possible to have a smaller incremental area under the curve between two tolerance test responses, even if the peak plasma triglyceride concentration is the same, because a higher baseline would reduce the area above that baseline. Therefore, it would appear that prolonged standing does not reduce the lipemic response following feeding per se, but does induce a reduced overall lipid profile. In other words, prolonged standing did not reduce the post-feeding rise from a fasted triglyceride concentration, but it does attenuate the overall concentration of triglycerides before and after feeding.

There were no changes in relative or absolute fat oxidation, yet triglyceride concentration decreased with prolonged standing; this would suggest that triglyceride is not leaving the blood stream because of increased use of triglyceride for energy. Intracellular triglyceride is stored in adipose and muscle tissue to be used for energy when needed, and is transported from the blood stream to these tissues with the aid of lipase enzymes (Oscai et al., 1990; Zimmermann et al., 2004). It is possible that prolonged standing enhances a component of the storing capacity of these tissues, thus increasing the clearance of triglyceride from the blood without oxidizing it.

By nature of the study, it is difficult to illuminate the mechanisms responsible for the observed response to prolonged standing. There are, however, several speculative connections can be made. Although not directly measured in this study, it is possible that there is an increase in the function or activity of a lipase responsible for triglyceride clearance. Lipoprotein lipase is the rate limiting lipase responsible for the clearance of chylomicron triglycerides and very low density lipoprotein triglyceride in muscle (Wang & Eckel, 2009), and is known to decrease in the presence of sedentary behavior (Bey &

Hamilton, 2003). Muscle lipoprotein lipase is primarily found in slow oxidative muscle fibers, and reaches its highest levels with low intensity ambulatory activity the activation of postural muscles in rats (Hamilton et al., 1998). It is possible that the inactivation of postural muscles that accompanies prolonged sitting could lead to reduced muscle lipoprotein lipase activity relative to the prolonged standing, and impair the clearance of triglyceride from the blood to muscle for storage

Lipoprotein lipase is produced in subendothelial parenchymal cells before translocating and binding to the endothelium of capillaries distributed throughout muscle, heart, and adipose tissue (Braun & Severson, 1992). The enzyme's activity can be tissue specific (Semb & Olivecrona, 1986), so it may be possible that changes in blood flow and shear stress to lower extremity muscles may alter the activity of lipoprotein lipase in the surrounding capillaries. Prolonged periods of sitting can result in impaired vascular function in the lower extremities due to reductions in shear stress (Restaino et al., 2016). Although it is yet to be shown, it is possible that lower extremity endothelial dysfunction and reduced muscle lipoprotein lipase activity are the nexus to a decrease in plasma triglyceride seen in prolonged standing.

Others may point to insulin as the causative factor for response observed in the study, but it is not possible to be certain because insulin was not measured in this study. It has been shown, however, that two days of sedentary activity with a hypercaloric diet can reduce whole body insulin sensitivity compared to an active and eucalorically fed group (Kim et al., 2016). In this study, the insulin explained 28% of the variation in postprandial triglyceride. Because sedentary-induced insulin resistance is limited to the area of the inactive muscle, it is possible that the full day of prolonged sitting in the present study reduced the insulin sensitivity of lower extremity muscles (Krogh-Madsen et al., 2010). If this is true, then excess nutrients may be unable to enter resistant muscle

tissue and instead be transported to the liver to be assembled into very low density lipoproteins (Petersen et al., 2007). It should be noted however, that there were no changes in glucose or fat oxidation, which are measures directly impacted by insulin. Even then, localized insulin resistance may not be shown through the calculation of insulin resistance because systemic plasma glucose and insulin may not be reflective of the lower extremity muscle, only (Seider et al., 1982).

The present study is not without limitations. Because insulin and lipoprotein lipase were not directly measured, it is difficult to know what mechanism is responsible for metabolic responses observed in the study. Because taller subjects exhibited a higher rate of energy expenditure, it is possible that taller subjects had some level of metabolic perturbation caused by spending more calories that would be alleviated with a more homogenous subject height. Some subjects experienced shifting pressures at work and in their lives, which prevented complete replication of control day steps. However, there was not a statistically significant difference and the standing trial is the one that displayed a lower step count on the second control day, which would effectively bias results against what was observed for standing if it did cause any change. Because subjects did not come in to the laboratory for a period to familiarize themselves with the procedures of obtaining gas samples, some hyperventilated resulting in an abnormally high respiratory exchange ratio. Although statistical analysis of gas samples obviated hyperventilating subjects, some may have been overfed on their intervention day as a result of this. Finally, one subject was obese and displayed fasted plasma triglyceride and glucose concentrations consistent with obesity, however, no statistical analysis performed gained or lost significance with the removal of this subject's data.

To conclude, the data show that a full day of prolonged standing does not alter the postprandial response to feeding, but does attenuate the total triglyceride lipid profile

compared to a day of prolonged sitting. Furthermore, prolonged standing does not induce a change in plasma glucose, relative or absolute fat oxidation. The findings of the study would suggest that posture does have an influence over metabolism independent of movement or activity.

TABLES AND FIGURES:

Table 1 Subject Characteristics

	Weight (kg)	Height (cm)	BMI
Sub 2	67.4	167.64	23.983
Sub 3	72.1	172.72	24.169
Sub 4	70.91	156	29.138
Sub 5	65.42	167.64	23.279
Sub 6	67.15	167.64	23.894
Sub 7	94.85	175.26	30.88
Sub 8	56.8	152.92	24.29
Sub 9	66.75	162.56	25.259
Sub 10	91.2	187.96	25.815
Sub 11	59.9	170.18	20.683
Sub 12	71.3	185	20.833
Sub 13	127.27	200.66	31.609
Sub 14	54.85	172.72	18.386
Sub 15	75.7	180.34	23.276
Sub 16	74	170.18	25.551
Sub 17	79.55	182.88	23.785
Sub 18	104.3	187.96	29.523
Sub 19	123.55	177.8	39.082
Mean	**79.06**	**174.3**	**25.75**
SEM	**± 4.988**	**± 2.805**	**± 1.137**

Weight, height, and BMI of subjects collected during preliminary testing

Table 2 Preliminary Testing

	Seated Metabolic Rate (kcal/day)	Standing Metabolic Rate (kcal/day)	Difference (kcal/day)
Sub 2	1734.126	1781.637	47.511
Sub 3	1941.583	1963.237	21.654
Sub 4	2052.24	2129.658	77.417
Sub 5	1744.702	1744.81	0.108
Sub 6	1591.073	1620.838	29.764
Sub 7	1932.708	2048.101	115.393
Sub 8	1686.235	1691.187	4.952
Sub 9	1972.356	2000.404	28.048
Sub 10	2227.042	2509.916	282.874
Sub 11	1805.852	1820.802	14.949
Sub 12	1887.402	2119.988	232.585
Sub 13	2825.495	3176.881	351.385
Sub 14	1832.164	1848.327	16.162
Sub 15	2253.332	2279.959	26.627
Sub 16	2832.254	2968.338	136.083
Sub 17	2240.512	2328.513	88.000
Sub 18	2976.802	3206.258	229.456
Sub 19	2591.942	2651.653	59.710
Mean	**2118**	***2216**	**97.93**
SEM	**±100.5**	**±117.8**	**±25.07**

Metabolic rates were measured through indirect calorimetry. Measurements were extrapolated to estimate full day metabolism . *denotes a significant difference from the seated metabolic rate (p=0.0011)

Table 3 Intervention Food

	Calories	Fat g	CHO g	PRO g	Fat %	CHO %	PRO %
Sub 2	1830	77	205.5	69.5	21.875	58.380	19.744
Sub 3	1940	83	212	70	22.739	58.082	19.178
Sub 4	2035	77	270	64.5	18.712	65.613	15.674
Sub 5	1745	65	173	70.5	21.069	56.077	22.853
Sub 6	1610	71	164	74	22.977	53.074	23.948
Sub 7	1940	83	212	70	22.739	58.082	19.178
Sub 8	1700	65	167	69	21.594	55.481	22.924
Sub 9	1985	83	218	71.5	22.281	58.523	19.195
Sub 10	2235	88	262	80.5	20.441	60.859	18.699
Sub 11	1805	76	191	69.5	22.585	56.760	20.654
Sub 12	1875	77	211.5	71	21.418	58.831	19.749
Sub 13	2815	109	347.5	100	19.58	62.443	17.969
Sub 14	1830	77	205.5	69.5	21.875	58.380	19.744
Sub 15	2250	88	264	81	20.323	60.969	18.71
Sub 16	2830	96	349.5	100.5	17.582	64.010	18.407
Sub 17	2235	88	262	80.5	20.441	60.859	18.699
Sub 18	2970	120	360	112	20.270	60.810	18.918
Sub 19	2720	103	343	103	18.761	62.477	18.761
Mean	**2131**	**84.78**	**245.4**	**79.25**	**20.96**	**59.43**	**19.61**
SEM	**±100.7**	**±3.438**	**±15.52**	**±3.399**	**±0.369**	**±0.739**	**±0.462**

Contents of food fed to subjects on their sitting/standing intervention day

Table 4 HFTT Test Meal

	Weight kg	Calories	Fat g	Sat Fat g	CHO g	PRO g	g/kg fat
Sub 2 Sit	67.4	1168.8	89.856	51.416	84	16	1.3332
Sub 3 Sit	72.1	1225.2	96.624	55.364	84	16	1.3401
Sub 4 Sit	70.91	1130.92	94.110	55.5644	72	12	1.3272
Sub 5 Sit	65.42	1065.04	86.205	50.9528	72	12	1.3177
Sub 6 Sit	67.15	1165.8	89.496	51.206	84	16	1.3328
Sub 7 Sit	94.85	1418.2	129.384	74.474	64	12	1.3641
Sub 8 Sit	56.8	1041.6	74.592	42.512	84	16	1.3132
Sub 9 Sit	66.61	1079.32	88.7184	50.7524	64	12	1.3319
Sub 10 Sit	91	1372	123.04	72.44	72	12	1.3521
Sub 11 Sit	59	988	76.96	45.56	72	12	1.3044
Sub 12 Sit	71.3	1135.6	95.472	54.692	64	12	1.3390
Sub 13 Sit	127.27	1807.24	176.069	101.7068	64	12	1.3834
Sub 14 Sit	54.85	938.2	71.784	40.874	64	12	1.3087
Sub 15 Sit	74.7	1176.4	100.368	57.548	64	12	1.3436
Sub 16 Sit	74	1248	99.36	56.96	84	16	1.3427
Sub 17 Sit	78.6	1223.2	105.184	62.024	72	12	1.3382
Sub 18 Sit	104.3	1531.6	142.192	83.612	72	12	1.3633
Sub 19 Sit	123.55	1762.6	169.912	99.782	72	12	1.3752
Sub 2 Stand	67.85	1174.2	90.504	51.794	84	16	1.3339
Sub 3 Stand	71.95	1223.4	96.408	55.238	84	16	1.3399
Sub 4 Stand	70.91	1130.92	94.1104	55.5644	72	12	1.3272
Sub 5 Stand	65.9	1070.8	86.896	51.356	72	12	1.3186
Sub 6 Stand	68.65	1183.8	91.656	52.466	84	16	1.3351
Sub 7 Stand	85.8	1309.6	116.352	66.872	64	12	1.3561
Sub 8 Stand	57.5	1050	75.6	43.1	84	16	1.3148
Sub 9 Stand	66.75	1081	88.92	50.87	64	12	1.3321
Sub 10 Stand	91.2	1374.4	123.328	72.608	72	12	1.3523
Sub 11 Stand	59.9	998.8	78.256	46.316	72	12	1.3064
Sub 12 Stand	71.35	1136.2	95.544	54.734	64	12	1.3391
Sub 13 Stand	128.95	1827.4	178.488	103.118	64	12	1.3842
Sub 14 Stand	55.65	947.8	72.936	41.546	64	12	1.3106
Sub 15 Stand	75.7	1188.4	101.808	58.388	64	12	1.3449
Sub 16 Stand	74.65	1248	99.36	56.96	84	16	1.3310
Sub 17 Stand	79.55	1234.6	106.552	62.822	72	12	1.3394
Sub 18 Stand	106.1	1553.2	144.784	85.124	72	12	1.3646
Sub 19 Stand	124.1	1769.2	170.704	100.244	72	12	1.3755
Mean	**78.95**	**1249**	**106.2**	**61.57**	**72.67**	**13.11**	**1.339**
SEM	**±3.487**	**±40.11**	**±4.994**	**±2.947**	**±1.319**	**±0.303**	**±0.00355**

Contents of the test meal ingested during the HFTT

Table 5 Steps

	Sit Control Day 1	Sit Control Day 2	Sit Intervention Day	Stand Control Day 1	Stand Control Day 2	Stand Intervention Day
Sub 2	3996	9366	1392	9052	7002	2538
Sub 3	10242	8942	1842	6738	4918	1822
Sub 4	7876	10330	4962	8516	10242	2494
Sub 5	4500	6206	2216	7378	3622	4142
Sub 6	12090	11984	7072	5204	9148	6818
Sub 7	14522	9418	3160	6980	9040	4510
Sub 8	6789	6124	1868	8550	8434	1122
Sub 9	7744	7996	1198	8910	7662	1490
Sub 10	16328	5258	2268	18588	4238	4488
Sub 11	6304	7536	730	5760	6982	654
Sub 12	4096	11948	2524	4096	1976	1986
Sub 13	9600	8690	2902	7270	7414	2954
Sub 14	2242	6014	3940	6402	3698	6180
Sub 15	8221	8819	1897	9514	7952	2074
Sub 16	5242	4784	6526	4312	5238	6836
Sub 17	7568	6020	782	7914	5314	6294
Sub 18	10740	7318	1414	9107	8516	4772
Sub 19	5830	6170	1612	7630	3634	2756
Mean	**7996**	**7940**	**2684**	**7885**	**6391**	**3552**
SEM	**±880.7**	**±508.8**	**±433.6**	**±736.1**	**±558**	**±474.3**

Steps Taken during the two control days as well as the intervention day. There was no significant difference in the number of steps taken between sit/stand control days ($p=0.117$) or sit/stand intervention days ($p=0.053$).

Table 6 Standing Time (hours)

	Sit Control Day 1	Sit Control Day 2	Sit Intervention Day	Stand Control Day 1	Stand Control Day 2	Stand Intervention Day
Sub 2	2.438	2.43	0.52	4.59	3.11	12.26
Sub 3	3.692	4.43	0.99	1.886	5.69	11.7
Sub 4	4.186	6.64	1.43	3.266	2.23	12.29
Sub 5	1.397	2.97	1.174	2.689	1.97	13.12
Sub 6	1.639	4.953	0.675	7.06	1.61	12.07
Sub 7	1.73	2.628	1.08	2.71	4.57	13.36
Sub 8	2.736	2.021	0.698	3.679	5.9	12.16
Sub 9	6.467	2.97	0.909	2.184	6.14	12.35
Sub 10	1.614	2.73	0.843	2.37	1.29	12.21
Sub 11	2.05	2.83	0.54	1.698	3.99	12.14
Sub 12	1.127	1.72	0.91	2.097	3.71	12.21
Sub 13	1.774	1.74	1	1.0975	2.5	12.25
Sub 14	0.79	1.9	0.9867	0.916	2.41	11.47
Sub 15	2.151	2.03	0.67	2.352	1.68	11.69
Sub 16	2.8	2.74	0.62	2.214	4.77	12.19
Sub 17	1.707	1.18	0.664	1.959	1.02	12.085
Sub 18	1.83	1.74	0.48	1.121	1.18	12.08
Sub 19	2.19	1.78	0.477	1.64	1.05	12.36
Mean	**2.351**	**2.746**	**0.8148**	**2.529**	**3.046**	***12.22**
SEM	**±0.313**	**±0.320**	**±0.0625**	**±0.343**	**±0.414**	**±0.105**

Time spent standing during the two control days as well as the intervention day. There were no significant differences in standing time between sit/stand control days ($p=0.379$). (*) Significantly greater time spent standing compared to the time spent standing on the sit intervention day ($p<0.001$)

Table 7 Intervention Day Posture (hours)

	Sit Trial Sit Time	Stand Trial Sit Time	Sit Trial Stand Time	Stand Trial Stand Time	Sit Trial Stepping Time	Stand Trial Stepping Time
Sub 2	13.515	2.74333	0.82	12.26	0.3	0.48
Sub 3	13.455	1.1	0.99	11.7	0.42	0.47
Sub 4	15.12	2.588	1.43	12.29	1.028	0.467
Sub 5	13.325	0.8567	1.174	13.12	0.397	0.96
Sub 6	13.11	0.845	0.675	12.07	0.626	1.13
Sub 7	16.297	3.95	1.08	13.36	0.338	1.01
Sub 8	12.84	1.31	0.698	12.16	0.178	0.171
Sub 9	14.78	1.087	0.909	12.35	0.282	0.2
Sub 10	15.25	3.86	0.843	12.21	0.44	0.85
Sub 11	16.27	2.55	0.54	12.14	1.32	0.19
Sub 12	15.62	2.27	0.91	12.21	0.47	0.52
Sub 13	13.35	3.45	1	12.25	0.65	0.64
Sub 14	13.32	3.757	0.9867	11.47	0.557	1.17
Sub 15	14.22	1.82	0.67	11.69	0.51	0.49
Sub 16	13.31	1.322	0.62	12.19	1.07	1.22
Sub 17	14.668	3.61	0.664	12.085	0.23	1.28
Sub 18	15.203	3.477	0.48	12.08	0.35	1.01
Sub 19	16.405	1.408	0.477	12.36	0.37	0.53
Mean	**14.45**	***2.334**	**0.8315**	**#12.22**	**0.5298**	**0.7104**
SEM	**±0.286**	**±0.269**	**±0.0600**	**±0.105**	**±0.0735**	**±0.0876**

Time spent sitting, standing, or stepping on the day of intervention. (*) Significant difference in sitting time between trials ($p<0.001$). (#) Significant difference stand time between trials ($p<0.001$).

Table 8 HFTT Metabolic Rate (kcal/hour)

	Sit BL	Sit H2	Sit H4	Sit H6	Stand BL	Stand H2	Stand H4	Stand H6
Sub 2	68.181	82.105	75.800	73.518	72.55	81.645	76.010	79.893
Sub 3	85.436	95.297	96.744	105.904	93.457	97.035	94.863	95.792
Sub 5	70.149	72.892	75.207	72.179	71.476	76.006	71.483	70.514
Sub 8	67.465	77.420	71.429	78.834	61.669	76.817	71.752	70.37
Sub 9	92.442	101.71	96.136	94.868	96.841	73.031	97.663	90.479
Sub 11	64.274	79.668	77.512	67.830	65.013	83.176	54.616	57.324
Sub 12	93.672	105.914	95.829	98.659	92.079	112.871	103.704	103.960
Sub 13	109.69	139.918	129.839	132.263	112.544	123.174	123.236	119.212
Sub 14	67.439	86.135	78.453	81.272	69.397	78.988	75.204	76.553
Sub 15	74.408	94.96	93.218	87.100	77.358	96.793	84.017	98.08
Sub 17	84.754	98.156	97.061	100.158	83.239	86.502	95.713	88.987
Sub 19	89.688	111.202	108.153	104.621	84.052	84.805	87.084	89.926
Mean	**80.63**	**95.45**	**91.28**	**91.43**	**81.64**	**89.24**	**86.28**	**86.76**
SEM	**±4.078**	**±5.316**	**±4.869**	**±5.309**	**±4.328**	**±4.478**	**±5.243**	**±4.876**

Metabolic rate during the HFTT, measured through indirect calorimetry

Table 9a HFTT Relative Fat Oxidation (%)

	Sit BL	Sit H2	Sit H4	Sit H6	Stand BL	Stand H2	Stand H4	Stand H6
Sub 2	48.402	49.105	82.273	80.345	56.011	36.725	58.620	72.881
Sub 3	71.630	35.543	72.895	74.310	77.378	59.232	85.267	87.919
Sub 5	79.649	54.898	55.442	76.590	62.250	57.895	63.116	71.660
Sub 8	33.943	42.545	66.721	85.875	52.854	35.151	47.046	70.654
Sub 9	67.882	55.008	83.113	79.521	83.181	58.058	71.651	80.767
Sub 11	29.098	31.455	33.286	54.847	56.613	30.173	62.069	19.704
Sub 12	68.702	52.156	88.477	89.112	68.840	86.233	100	100
Sub 13	76.57	46.264	98.545	100	86.151	72.519	100	100
Sub 14	64.24	58.717	79.589	85.826	83.580	49.097	84.076	89.444
Sub 15	70.594	76.66	100	100	73.92	62.054	76.353	76.639
Sub 17	100	89.383	100	100	66.448	71.651	93.275	100
Sub 19	41.825	69.281	70.136	88.481	77.83	98.195	100	100
Mean	**62.71**	**55.08**	**77.54**	**84.58**	**70.42**	**59.75**	**78.46**	**80.81**
SEM	**±5.951**	**±4.828**	**±5.686**	**±3.743**	**±3.361**	**±5.916**	**±5.24**	**±6.513**

Table 9b HFTT Absolute Fat Oxidation (kcal/hour)

	Sit BL	Sit H2	Sit H4	Sit H6	Stand BL	Stand H2	Stand H4	Stand H6
Sub 2	33.001	40.318	62.363	59.068	40.636	29.984	44.557	58.227
Sub 3	61.198	33.871	70.521	78.698	72.316	57.476	80.888	84.219
Sub 5	55.873	40.016	41.696	55.282	44.494	44.004	45.117	50.530
Sub 8	22.900	32.938	47.658	67.699	32.595	27.002	33.757	49.721
Sub 9	62.752	55.952	79.902	75.440	80.553	42.400	69.977	73.077
Sub 11	18.702	25.060	25.801	37.203	36.806	25.097	33.899	11.295
Sub 12	64.355	55.241	84.787	87.917	63.388	97.332	103.704	103.96
Sub 13	83.998	64.732	127.952	132.263	96.958	89.325	123.236	119.21
Sub 14	43.326	50.576	62.440	69.753	58.003	38.780	63.228	68.472
Sub 15	52.527	72.796	93.218	87.100	57.190	60.064	64.149	75.172
Sub 17	84.754	87.735	97.060	100.158	55.310	61.979	89.277	88.987
Sub 19	37.513	77.043	75.854	92.571	65.417	83.274	87.084	89.926
Mean	**51.74**	**53.02**	**72.44**	**78.6**	**58.64**	**54.73**	**69.91**	**72.73**
SD	**±6.195**	**±5.649**	**±7.92**	**±7.041**	**±5.433**	**±7.122**	**±8.126**	**±8.192**

Relative and absolute fat oxidation during the HFTT, measured through indirect calorimetry. % energy from fat is relative to % energy from carbohydrate.

Table 10 HFTT Postprandial Energy Expenditure (kcal/6h)

	Sitting	Standing
Sub 2	462.848	475.099
Sub 3	595.892	575.383
Sub 5	440.557	436.008
Sub 8	455.367	437.884
Sub 9	585.443	522.347
Sub 11	450.022	390.233
Sub 12	600.807	641.071
Sub 13	804.044	731.247
Sub 14	491.724	461.490
Sub 15	550.558	557.793
Sub 17	590.750	542.406
Sub 19	647.955	523.631
Mean	**556.3**	***524.5**
SEM	**±30.55**	**±27.54**

Postprandial energy expenditure measured after the ingestion of the test meal during the HFTT. (*) Significantly greater postprandial energy expenditure during the HFTTstand compared to HFTTsit (p=0.032).

Table 11a Sit Triglyceride (mM)

	Sit BL	Sit H1	Sit H2	Sit H3	Sit H4	Sit H5	Sit H6
Sub 2	0.6800	1.1306	1.2042	1.7411	1.0107	1.0441	0.8236
Sub 3	0.7251	1.1228	1.8569	2.3232	2.6615	2.5508	2.3668
Sub 4	1.7400	2.0010	2.0903	2.3706	2.3866	1.9717	1.3939
Sub 5	1.7338	2.2832	3.0414	2.7715	3.1355	2.3725	2.0238
Sub 6	0.8655	0.8008	1.2226	1.1994	1.2341	0.7332	0.8548
Sub 7	0.8333	1.2643	2.4142	2.6549	2.1256	1.4717	1.2494
Sub 8	1.1168	1.2472	1.9119	1.6300	1.6046	1.4983	1.4674
Sub 9	2.0120	2.1526	2.4402	2.4750	2.6234	2.5130	2.2216
Sub 10	0.8455	1.2340	1.7901	1.6969	1.5168	1.4485	1.2182
Sub 11	0.9659	1.2177	1.1159	1.4241	1.5808	1.1403	0.9568
Sub 12	0.8208	0.8535	1.3021	2.2022	2.6912	1.9628	1.4365
Sub 13	0.8637	1.0839	1.5799	1.8910	1.7910	1.8308	1.2803
Sub 14	0.7922	1.2502	2.2888	2.1708	1.8038	1.3931	1.1810
Sub 15	0.9888	1.1903	2.2135	3.7133	5.3204	4.9714	3.7811
Sub 16	1.3364	1.6260	2.6527	2.6293	2.7961	2.9844	2.7406
Sub 17	0.6224	1.0211	1.6090	1.9035	2.0230	1.5259	1.3705
Sub 18	1.1517	1.6341	2.6904	2.9723	3.5127	2.9921	3.2556
Sub 19	2.3783	2.4778	2.9510	3.4231	4.5513	4.7275	5.7789
Mean	**1.137**	**1.422**	**2.021**	**2.289**	**2.465**	**2.174**	**1.967**
SEM	**±0.1186**	**±0.117**	**±0.1434**	**±0.1582**	**±0.2653**	**±0.2747**	**±0.2984**

Table 11b. Stand Triglyceride (mM)*

	Stand BL	Stand H1	Stand H2	Stand H3	Stand H4	Stand H5	Stand H6
Sub 2	0.8917	1.4488	1.1982	1.2140	1.6090	1.4345	0.9169
Sub 3	1.0048	1.1531	1.8262	1.9566	2.0303	2.1403	1.6284
Sub 4	1.1043	1.4117	1.8641	1.9981	2.0368	1.4646	1.2604
Sub 5	1.5397	1.7413	2.2885	2.4599	2.1776	1.7830	1.8522
Sub 6	0.8491	0.8775	1.3008	1.4619	1.3220	0.7747	0.7154
Sub 7	0.5370	0.6805	1.1186	1.5595	1.4310	1.0115	1.2599
Sub 8	0.9432	1.1437	1.4674	1.6007	1.2673	1.3210	1.1789
Sub 9	1.6856	2.0394	2.0494	2.5733	2.3270	1.8548	1.7639
Sub 10	0.7235	0.9162	1.5226	1.5182	1.5126	1.0143	0.9207
Sub 11	1.0490	1.2775	1.3259	1.2989	1.8177	1.0181	0.8193
Sub 12	0.7249	0.8687	1.8194	2.5207	2.6054	2.2429	1.8408
Sub 13	0.7849	1.1789	2.4484	2.4635	2.3522	1.8620	1.1088
Sub 14	0.6580	0.8474	2.1255	2.0066	1.7642	1.4427	1.2560
Sub 15	1.5520	1.9718	1.2919	1.9244	2.5230	2.1672	1.3428
Sub 16	0.8699	1.1666	1.7838	2.9511	3.3458	3.5577	2.9950
Sub 17	0.7123	1.0512	1.9453	2.3846	1.7549	1.4309	1.1702
Sub 18	0.8785	1.2105	2.5162	3.5511	4.1318	3.3761	3.5044
Sub 19	2.0115	2.1977	2.5263	2.7624	3.4016	3.2062	3.7325
Mean	**1.029**	**1.288**	**1.801**	**2.123**	**2.19**	**1.839**	**1.626**
SEM	**±0.09513**	**±0.1035**	**±0.1091**	**±0.1489**	**±0.1847**	**±0.1949**	**±0.2108**

Plasma triglyceride concentration in mM throughout the HFTT. (*) Significantl effect of posture on plasma triglyceride concentration between the two trials (time p>.001, Intervention p=0.036).

Table 12. Triglyceride Total and Incremental AUC (mMx6h)

	Sit Total AUC	Stand Total AUC	Sit AUCi	Stand AUCi
Sub 2	6.8824	7.8096	2.8020	2.4586
Sub 3	12.0616	10.4240	7.7102	4.3944
Sub 4	12.3891	9.9576	4.0239	3.3316
Sub 5	15.4836	12.1520	5.0810	2.9081
Sub 6	6.0511	6.5198	1.6511	2.2271
Sub 7	10.9729	6.6994	5.9721	3.4773
Sub 8	9.1840	7.8615	2.4835	2.2023
Sub 9	14.3204	12.5698	2.2486	2.4552
Sub 10	8.7187	7.3059	3.6456	2.9646
Sub 11	7.4403	7.6729	1.6455	2.7568
Sub 12	10.1406	11.3388	5.2154	6.9908
Sub 13	9.2484	11.2519	4.0668	6.5424
Sub 14	9.8932	9.1433	5.1397	5.1962
Sub 15	19.7978	11.3275	13.8573	3.5744
Sub 16	14.7269	14.7382	6.7084	9.5183
Sub 17	9.0790	9.5081	5.3442	5.2346
Sub 18	16.0031	16.9744	9.0948	11.7115
Sub 19	22.2146	16.9631	7.9394	4.8969
Mean	**11.92**	***10.57**	**5.257**	**4.602**
SEM	**±1.042**	**±0.7591**	**±0.7207**	**±0.6225**

Plasma triglyceride total and incremental AUC from the HFTT. (*) standing total AUC is significantly lower than sitting (p=0.022) There is no significant difference between incremental areas under the curve (p=0.186)

Table 13a Sit Glucose

	Sit BL	Sit H1	Sit H2	Sit H3	Sit H4	Sit H5	Sit H6
Sub 2	3.5013	4.2368	4.9248	5.4478	5.5457	5.5540	4.0186
Sub 3	5.0626	3.9411	6.8046	6.8235	6.1874	7.4263	7.2625
Sub 4	4.9785	6.1302	4.8038	5.4191	5.6212	6.1985	4.6883
Sub 5	5.6023	5.7422	9.2564	6.6492	7.3558	6.4854	5.5130
Sub 6	6.5249	4.2988	5.5773	6.6015	6.8318	5.5226	5.1687
Sub 7	5.5316	6.2473	6.8490	7.9414	7.1848	6.0453	6.4283
Sub 9	5.0454	8.8512	7.0477	5.9420	6.3583	6.1346	5.7489
Sub 10	5.5796	5.1739	6.9278	7.5612	6.3583	6.2418	5.4206
Sub 11	5.1246	5.5796	7.4307	6.0447	5.7994	5.0514	4.8524
Sub 12	5.8498	5.1714	5.7989	6.8520	7.1219	7.3917	5.9408
Sub 13	5.4668	5.4413	5.9381	5.7405	5.6068	5.8726	5.3458
Sub 14	5.7688	6.6886	7.5412	7.8781	6.4083	5.9393	6.1091
Sub 15	4.8567	5.3246	5.0199	7.4202	7.8909	8.7192	5.7184
Sub 16	5.2885	5.4762	6.9495	6.2062	6.4166	6.4960	6.2495
Sub 17	5.9781	6.2756	5.6045	8.5231	8.2528	7.3530	6.8696
Sub 18	4.9224	5.4477	7.7316	6.7325	6.2817	6.7835	7.5068
Sub 19	5.9393	6.5005	8.5049	9.5981	10.9796	10.8393	11.0506
Mean	**5.354**	**5.678**	**6.63**	**6.905**	**6.835**	**6.709**	**6.111**
SEM	**±0.1595**	**±0.2742**	**±0.3074**	**±0.2754**	**±0.321**	**±0.3356**	**±0.3785**

Table 13b. Stand Glucose

	Stand BL	Stand H1	Stand H2	Stand H3	Stand H4	Stand H5	Stand H6
Sub 2	5.6467	6.4105	5.0871	3.4817	6.2035	6.3522	4.5905
Sub 3	5.3137	6.4999	6.3378	5.5773	6.0369	7.2687	5.6534
Sub 4	5.7516	4.5668	4.8661	6.2790	6.9417	6.6181	5.5027
Sub 5	6.0047	5.9870	7.5467	7.6300	7.2109	6.5715	5.7661
Sub 6	4.9037	6.0153	5.5912	5.9293	6.5904	5.7083	4.9567
Sub 7	4.8372	4.8813	6.5615	6.8868	5.8932	5.9737	5.901
Sub 9	5.6750	7.1021	10.4764	7.8148	7.6017	6.9251	5.7294
Sub 10	4.6193	4.0189	5.6817	5.9215	6.6592	5.3268	4.618
Sub 11	4.4996	5.4109	4.4981	5.1114	5.4566	5.0215	4.0788
Sub 12	5.7650	5.1930	6.5760	7.8635	8.1950	7.0987	5.6956
Sub 13	5.5796	5.1851	6.0808	7.5789	6.8779	6.7136	5.2073
Sub 14	4.3726	5.1385	6.8174	5.9870	5.3325	6.6675	5.2783
Sub 15	4.8950	4.6426	6.2079	9.5400	9.8002	8.7192	5.7184
Sub 16	4.4267	4.3331	5.3791	7.8437	7.8920	8.6919	6.9162
Sub 17	6.0863	6.8590	6.5348	8.9955	8.1118	7.1549	6.7313
Sub 18	5.7022	5.8643	5.824	8.9378	7.8415	8.9566	9.5134
Sub 19	5.9346	6.9646	7.5320	6.9167	6.3252	6.9983	5.9326
Mean	**5.295**	**5.593**	**6.329**	**6.959**	**6.998**	**6.869**	**5.752**
SD	**±0.1448**	**±0.2329**	**±0.3308**	**±0.378**	**±0.2787**	**±0.2701**	**±0.2919**

Plasma glucose concentration in mM throughout the HFTT. No significant differences were found.

Table 14. Glucose Total and Incremental AUC (mMx6h)

	Sit Total AUC	Stand Total AUC	Sit AUCi	Stand AUCi
Sub 2	29.46892	32.65505	8.45934	4.089791
Sub 3	37.34543	37.20666	8.5315	5.322057
Sub 4	33.00475	34.89755	3.263285	4.254648
Sub 5	41.04778	40.8313	7.443553	4.891874
Sub 6	34.68107	34.76433	4.93184	5.342595
Sub 7	40.24847	35.56364	7.060551	6.544332
Sub 9	39.73225	45.6216	9.458475	11.57331
Sub 10	37.76174	32.22764	4.815829	5.328163
Sub 11	34.892	29.79086	4.499436	2.978531
Sub 12	38.23355	40.65923	4.542732	6.877377
Sub 13	34.00388	37.82835	1.259465	5.012325
Sub 14	40.39279	34.76989	5.783879	8.5315
Sub 15	39.66009	44.21726	10.52422	15.14244
Sub 16	37.31213	39.80996	5.584052	13.3551
Sub 17	42.43547	44.06739	6.821869	7.549017
Sub 18	39.19383	45.03322	9.658302	10.81841
Sub 19	54.9191	40.67033	19.2833	5.062837
Mean	**38.49**	**38.27**	**7.172**	**7.216**
SEM	**1.232**	**1.105**	**0.9081**	**0.8022**

Total and Incremental areas under the curve for plasma glucose during the HFTT. No significant difference was found between total AUCs (p=0.433) or AUCi (p=0.485)

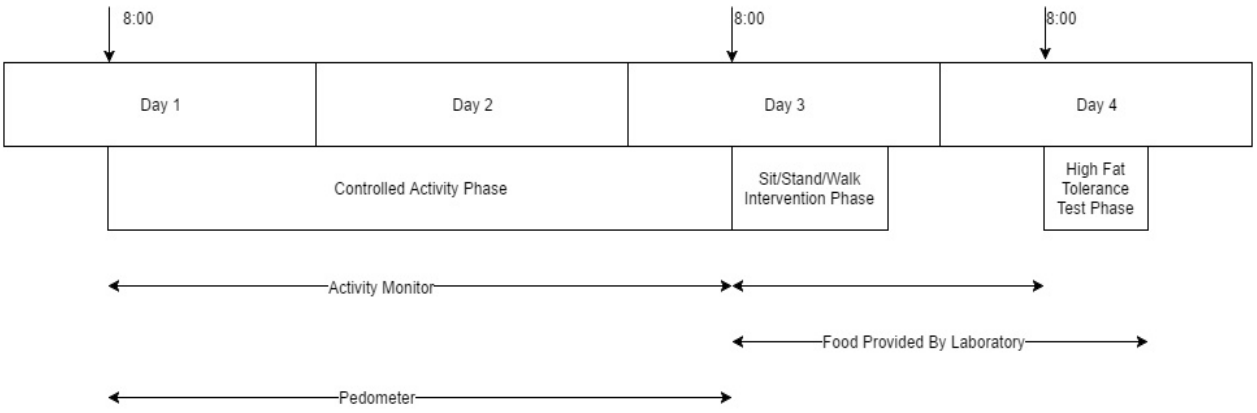

Figure 1. Experimental Design

Reactions:

1. TG --- Lipase ---> Glycerol + Fatty Acids
2. Glycerol + ATP --- GK---> G-3-P + ADP
3. G-3-P + O2 --- GPO---> DHAP + H_2O_2
4. H_2O_2 + TBHB --- Peroxidase--- > Quinoneimine dye + $2H_2O$

Figure 2. Chemical reactions used to spectrophotometrically measure plasma

Reactions:

1: Glucose + ATP ---- HK ---> G-6-P + ADP

2: G-6-P + NAD ---- G6PDH ---> 6-Phosphogluconate + NADH + H^+

Figure 3. Chemical reactions used to spectrophotometrically measure plasma glucose

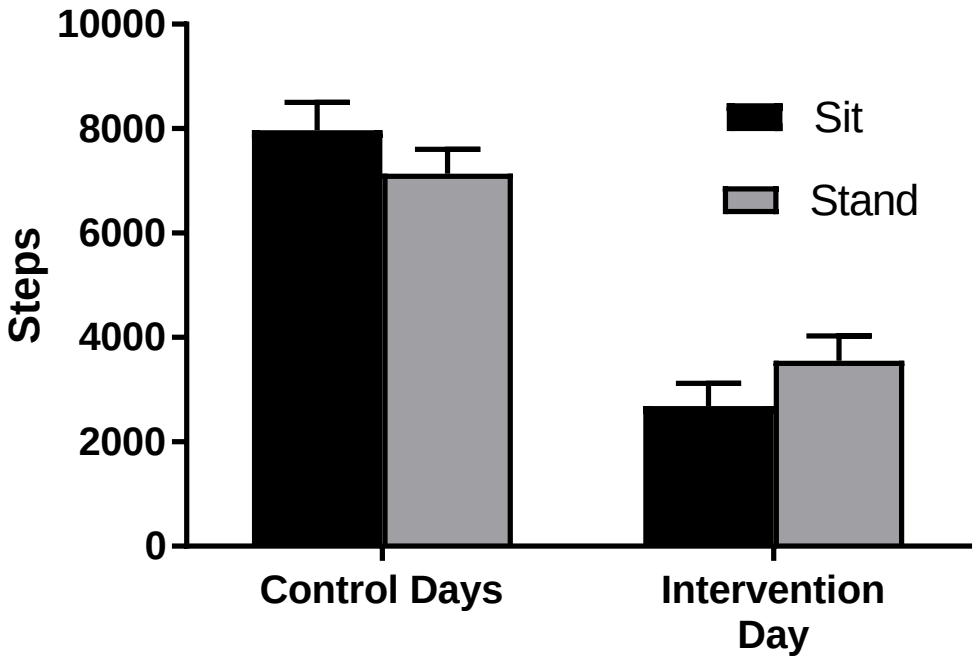

Figure 4. Steps taken during the two control days (averaged) and the intervention day. There was no significant difference in the number of steps that participants took on control days for the sitting trial compared to those for the standing trial ($p>0.05$). Likewise, there was no significant difference in the number of steps that participants took on the intervention day for the sitting trial compared to those for the standing trial ($p>0.05$).

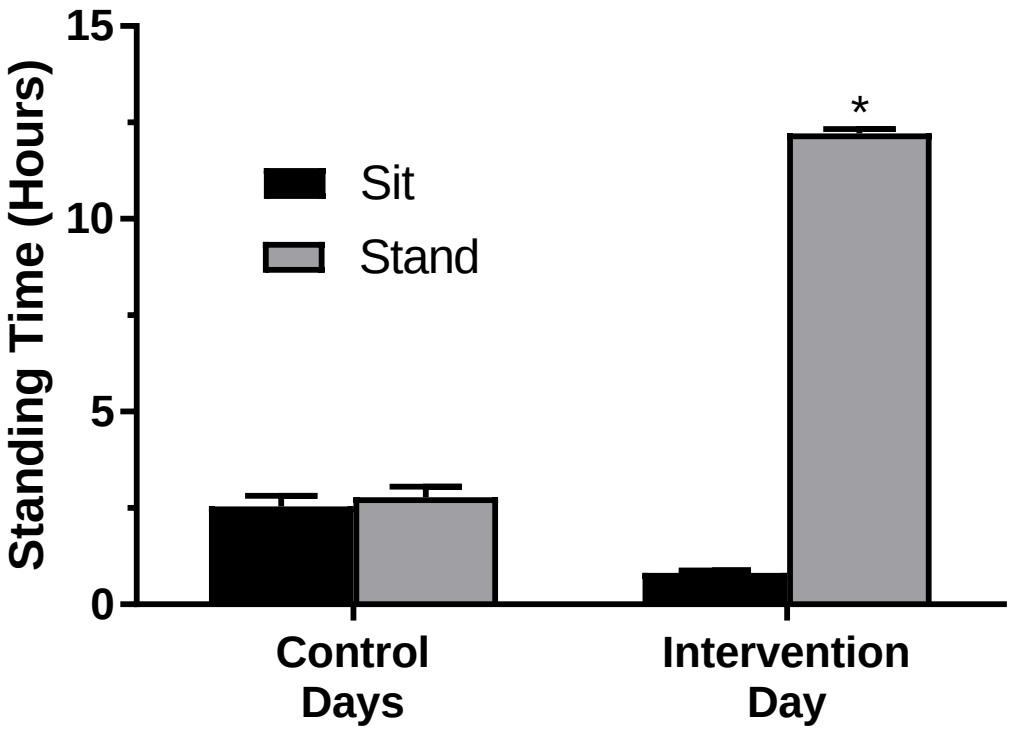

Figure 5. Time spent standing during the two control days (averaged) and intervention days. There was no difference in standing time during the control days between the sitting or the standing trial. (*) Significantly greater time spent standing on the standing intervention day compared to the sitting intervention day ($p<0.001$).

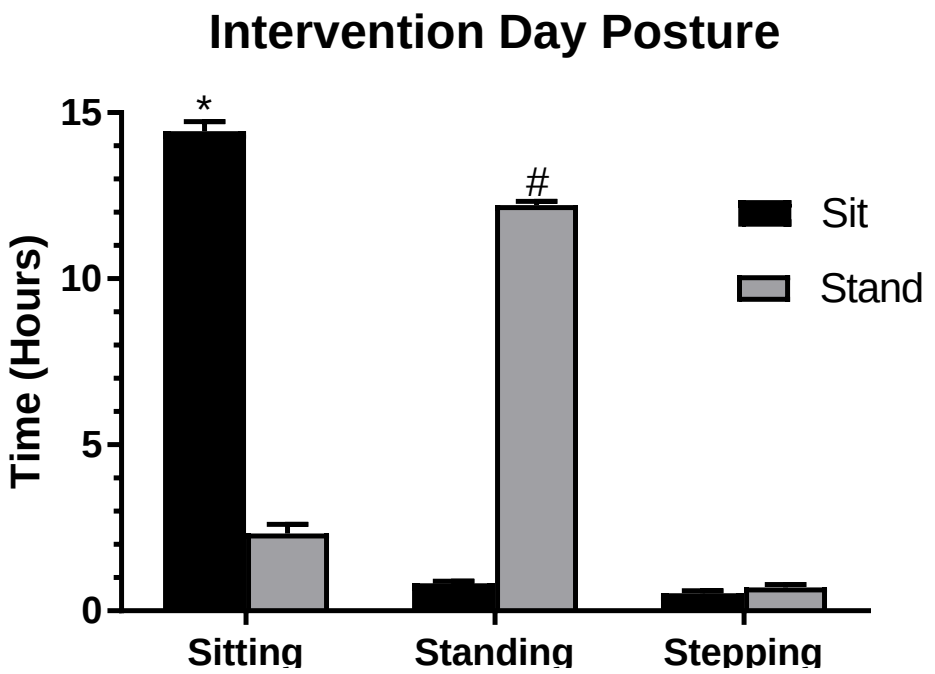

Figure 6. Time spent sitting, standing, and stepping during the intervention day. (*) Significantly greater time sitting compared to the stand trial ($p<.001$). (#) Significantly greater time standing compared to the sit trial. There was no difference in time spent stepping between the sit and stand trials ($p>0.05$).

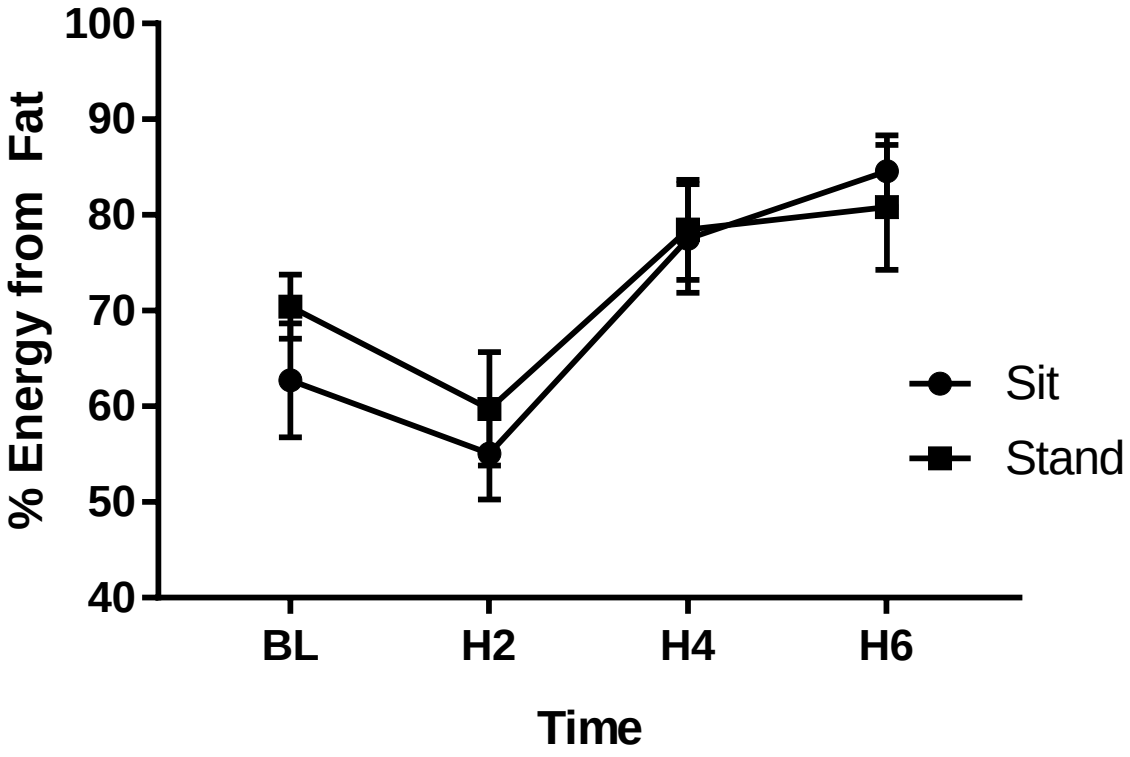

Figure 7. Relative fat oxidation during the HFTT. There was not a significant difference percentage of energy coming from fat between the two trials ($p>0.05$).

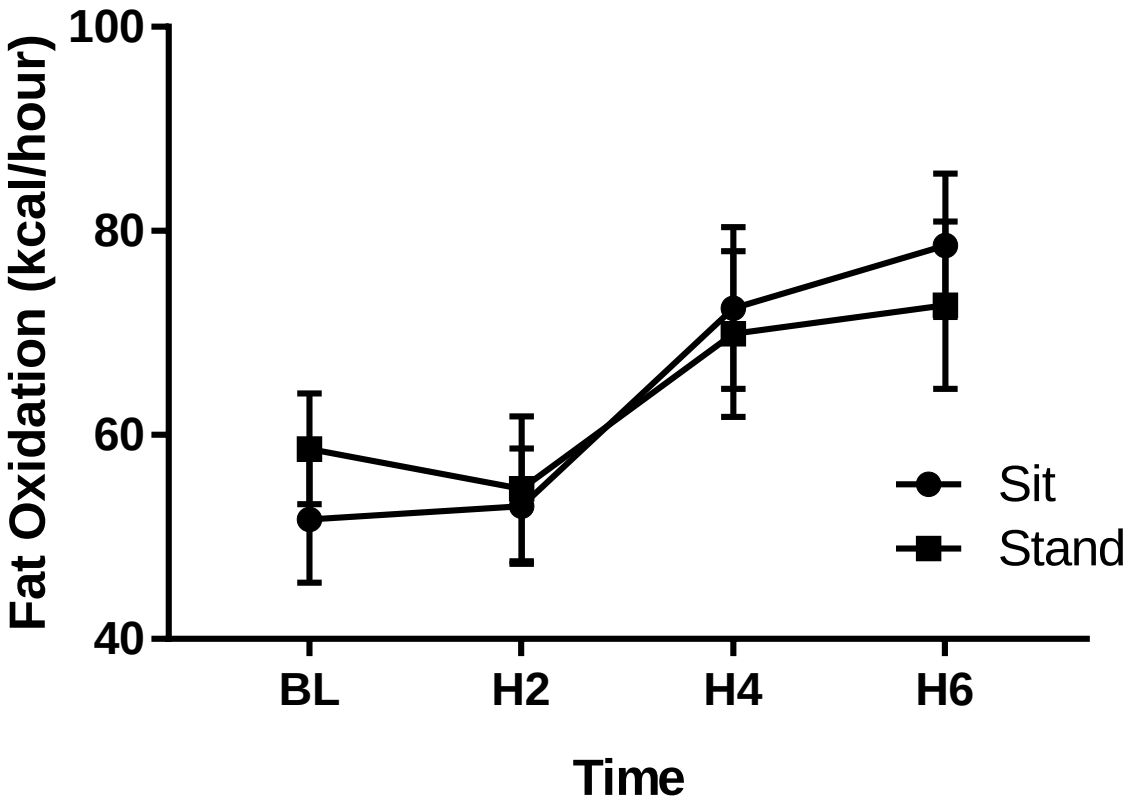

Figure 8. Absolute fat oxidation during the HFTT. There was no significant difference in fat oxidation between trials ($p>0.05$).

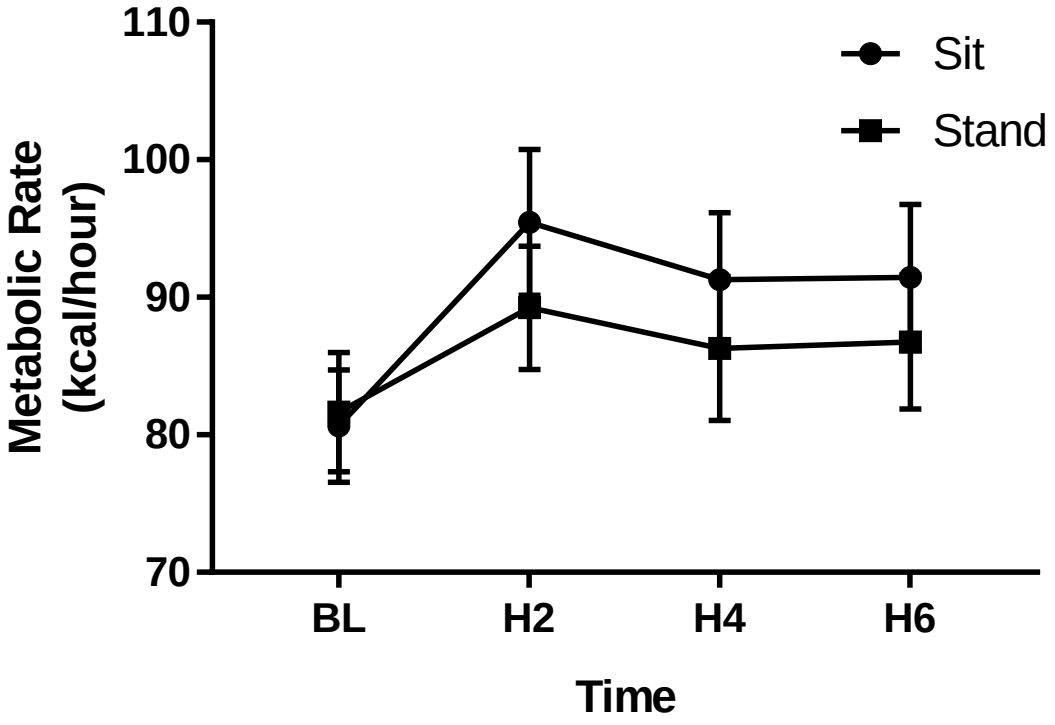

Figure 9. Metabolic rate measured by indirect calorimetry during the HFTT. There was no significant difference in metabolic rate between the two trials ($p>0.05$).

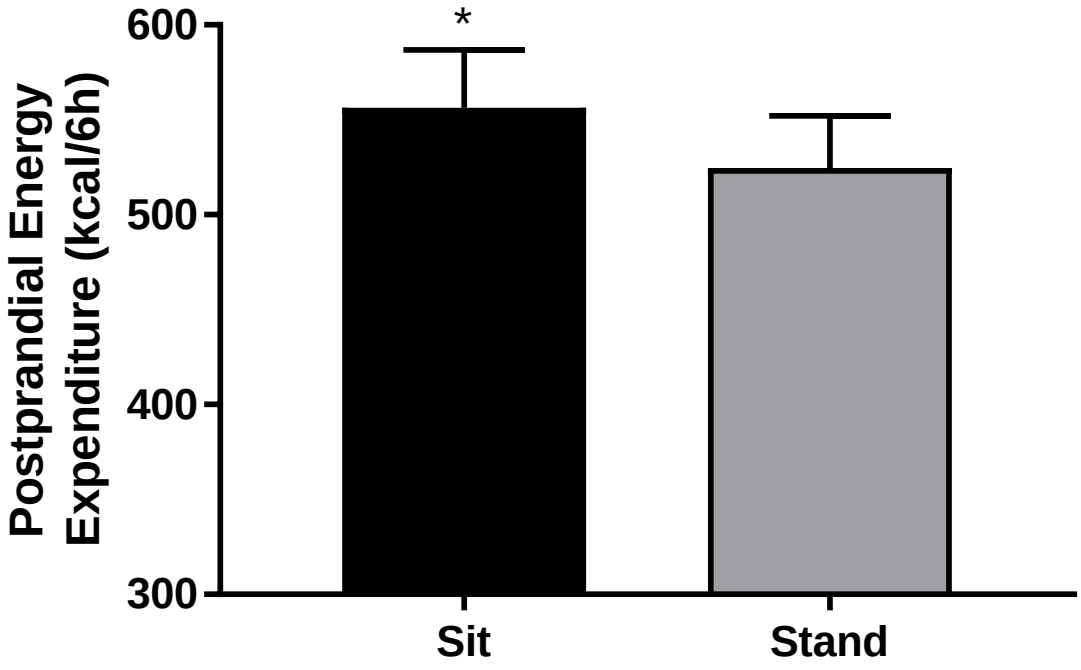

Figure 10. Measurements of postprandial energy expenditure during the HFTT. (*) Significantly greater postprandial energy expenditure compared to the standing trial (p=0.032).

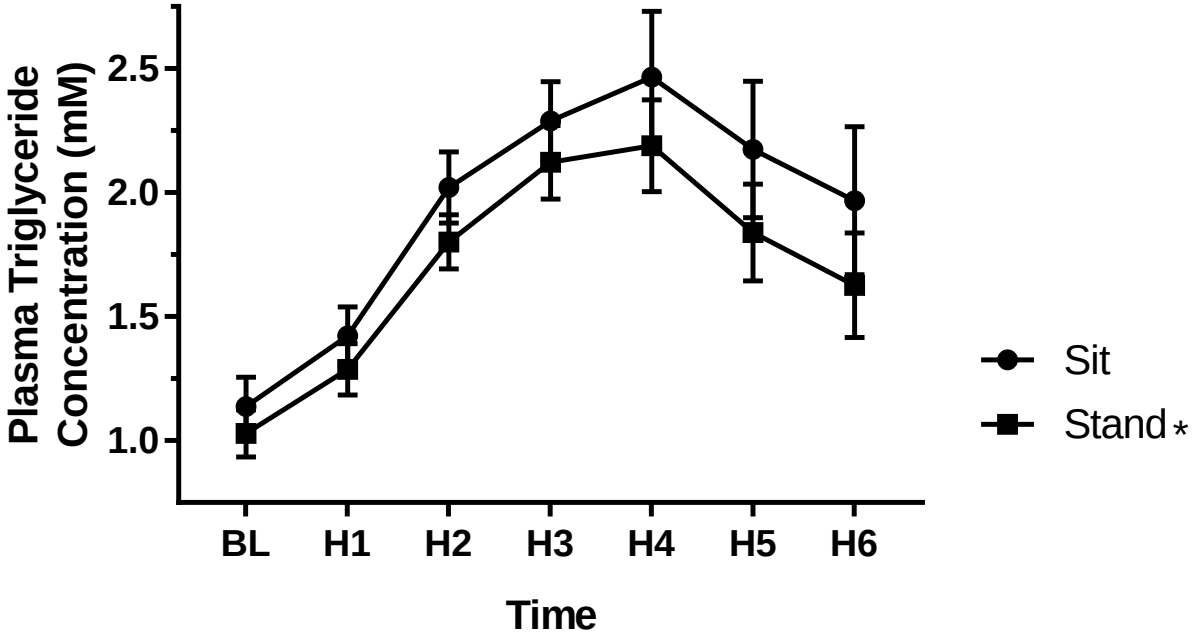

Figure 11. Plasma triglyceride concentration (mM) over the course of the HFTT was significantly lower in the standing trial compared to the sitting trial. (*) Significantly lower plasma triglyceride concentration than that in the sitting trial (p=0.036).

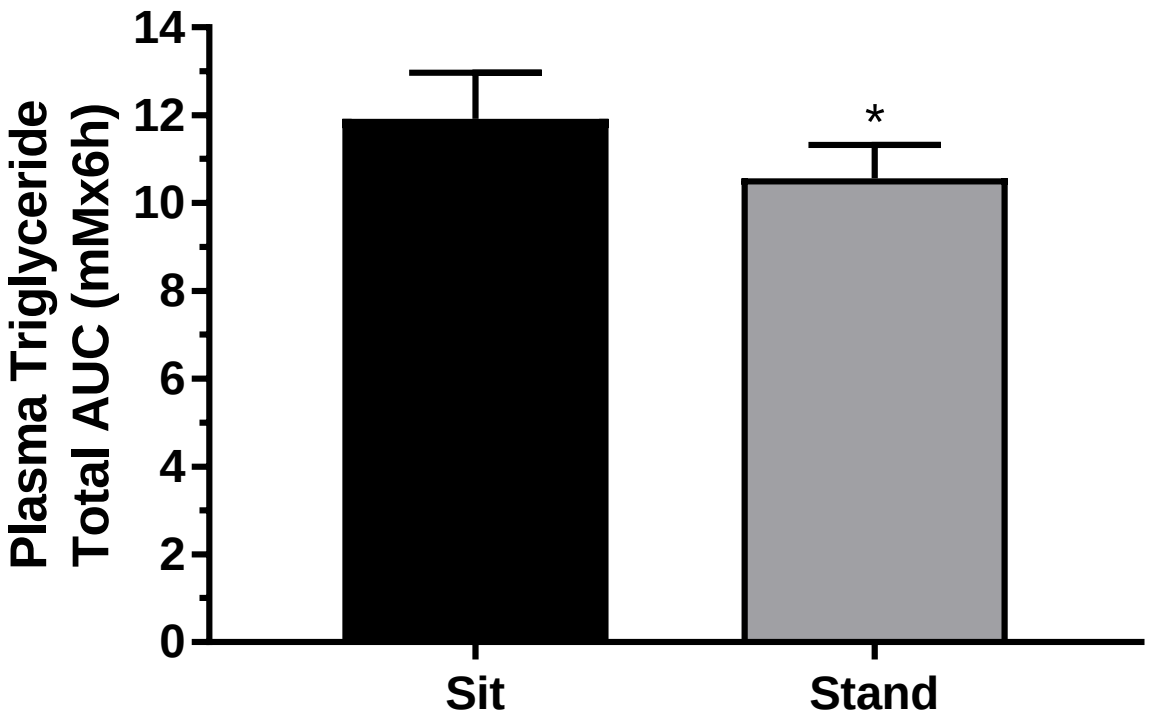

Figure 12. Total area under (AUC) the curve for plasma triglyceride during the high fat tolerance test. (*) Significantly lower total AUC for plasma triglyceride concentration over the course of the high fat tolerance test compared to sitting (p=0.022).

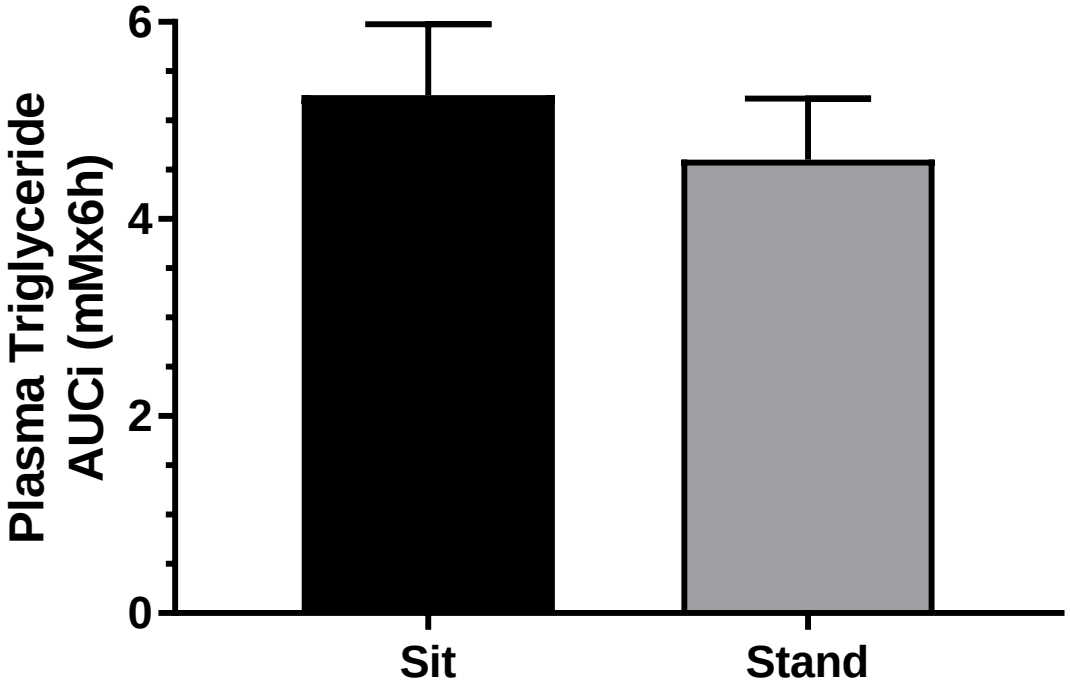

Figure 13. Incremental area under the curve (AUCi) for plasma triglyceride during the HFTT. There was no significant difference in AUCi for plasma triglyceride concentration over the course of the high fat tolerance test compared to sitting ($p>0.05$).

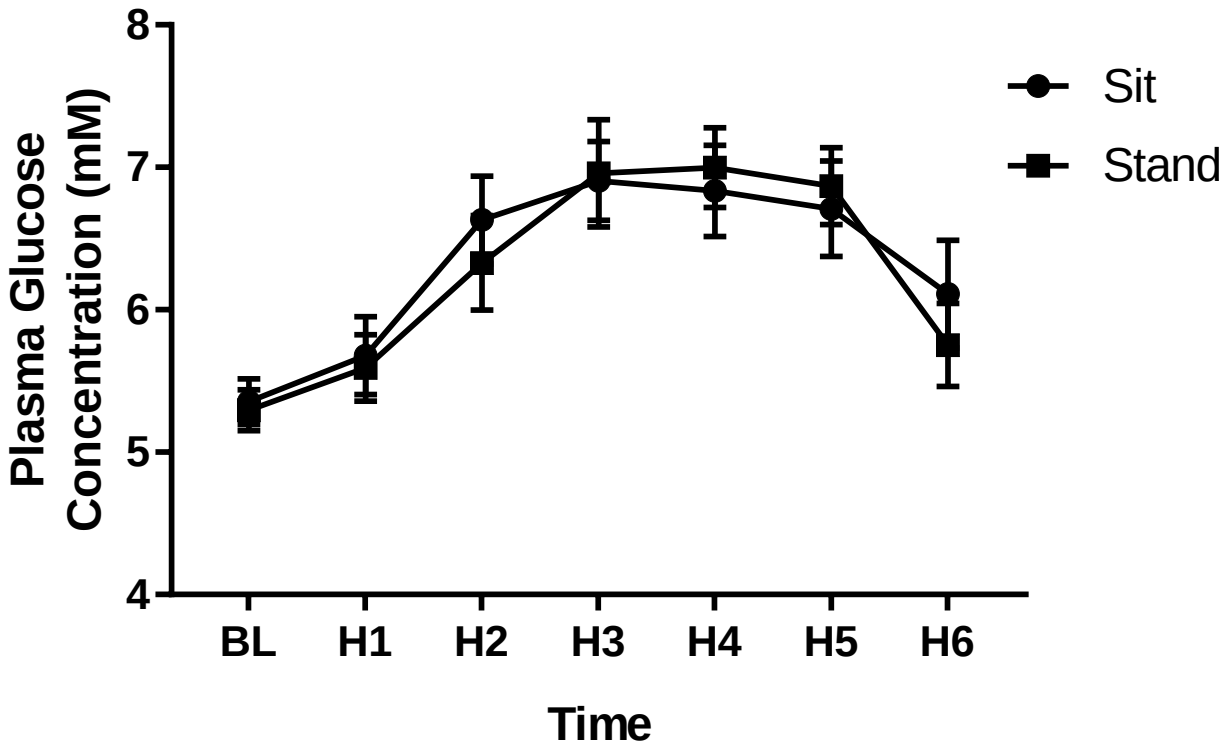

Figure 14. Plasma glucose concentration (mM) during the high fat tolerance test. There was no significant effect of posture on plasma glucose concentration ($p>0.05$).

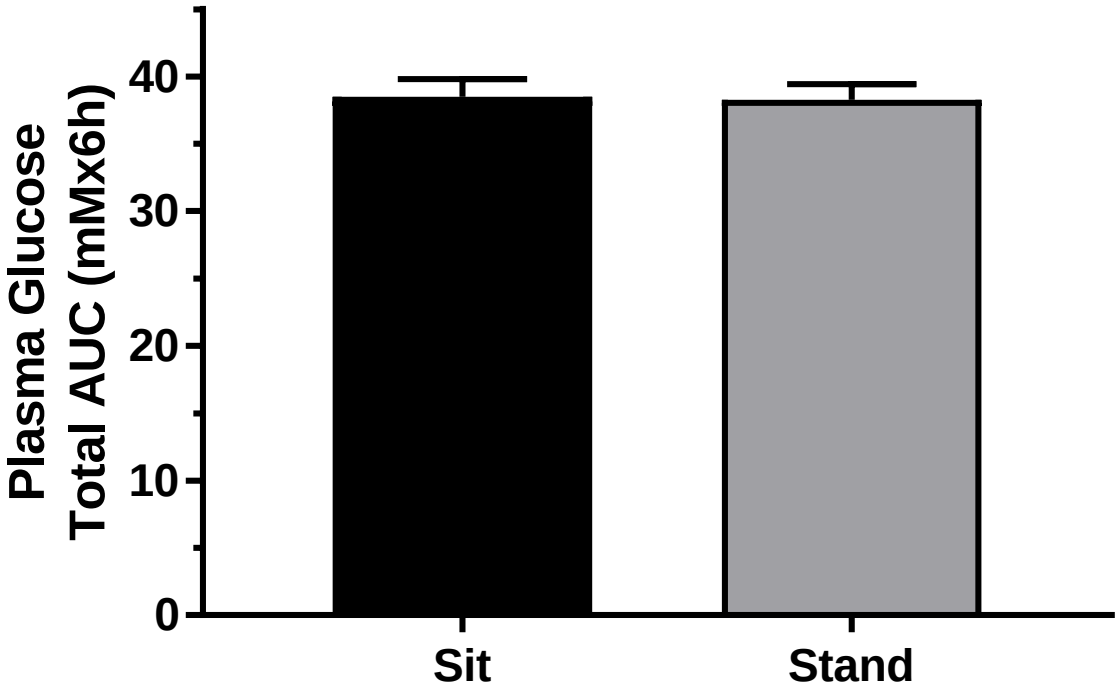

Figure 15. Total AUC for plasma glucose during the high fat tolerance test. There was no significant difference in AUC for plasma glucose concentration over the course of the high fat tolerance test compared to sitting ($p>0.05$).

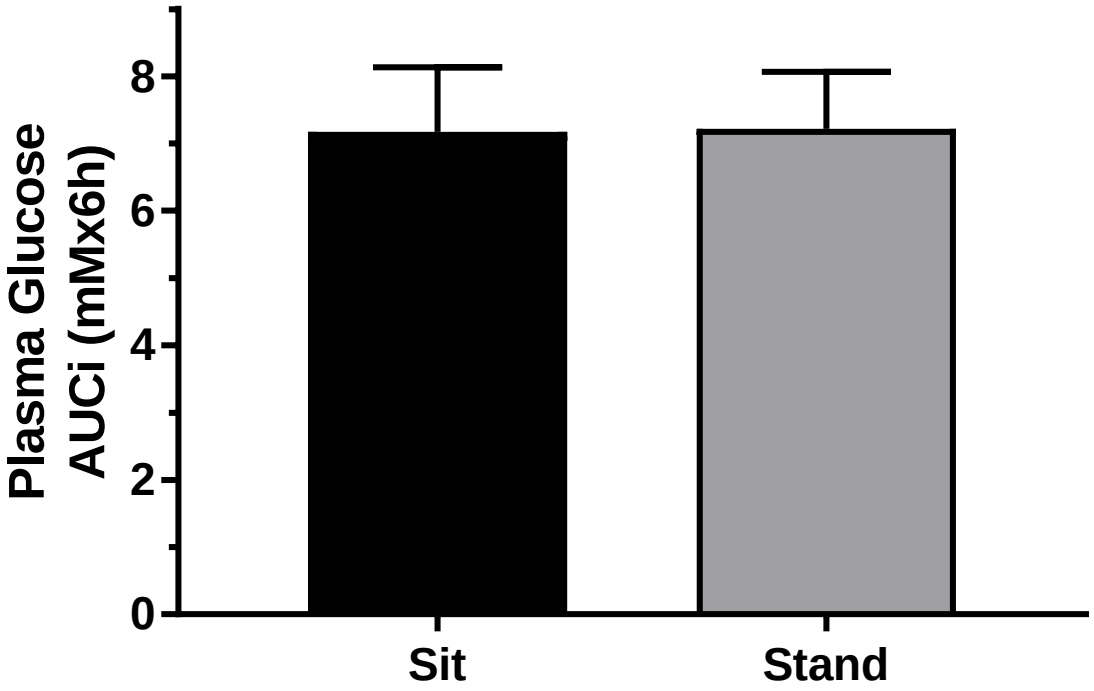

Figure 16. AUCi for plasma glucose during the high fat tolerance test. There was no significant difference in AUCi for plasma glucose concentration over the course of the high fat tolerance test compared to sitting ($p>0.05$).

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